Skeletal Muscle Metabolism, Morphology and Function in Master Athletes

By

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ABSTRACT

Skeletal muscle mass and function deteriorates with advancing age (sarcopenia). Physical inactivity and obesity are associated with advancing age and exacerbate inherent age-related decrements in physiological function, skeletal muscle mass and morphology. The study of older individuals that have chronically undertaken structured exercise training (Master Athletes; MA) provide an opportunity to elucidate the contribution of inherent ageing and biological ageing factors to age-related physiological deterioration. Chapter 2 describes the first systematic review to quantitatively and qualitatively summarise the literature surrounding MA. We established that, at present, there are few high-quality studies, utilising sophisticated measurement tools to comprehensively assess whether, and to what extent, physiological function and muscle morphology are enhanced/preserved in MA compared with trained and untrained older and younger individuals. Accordingly, in Chapter 3, we conducted a detailed mechanistic study to compare physical function, cardiorespiratory fitness, body composition, muscle strength, architecture and fibre-type morphology in MA, with healthy untrained younger and age-matched older individuals. We shed light on the morphological mechanisms through which chronic exercise training enhances skeletal muscle function and body composition, with implications for sarcopenia diagnosis. Based on the superior phenotype observed in MA, the final experimental in this thesis, Chapter 4, describes an investigation of in vivo skeletal muscle remodelling responsiveness in MA. This work demonstrates that basal and exercise-induced rates of muscle protein synthesis are equivalent between older untrained individuals and MA. As such, untrained older individuals retain a capacity to adapt to exercise and that it may never be too late to benefit from exercise training.

Keywords: Sarcopenia, Master Athlete, Skeletal Muscle, Muscle Protein Synthesis

"I think science has begun to demonstrate that ageing is a disease.

If it is, it can be cured."

Tom Robbins, Author (1932 - present)

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AUTHORS CONTRIBUTIONS

Chapter 2: James McKendry, Leigh Breen, Brandon J. Shad and Carolyn A. Greig conceived and designed the search strategy and protocol. James McKendry and Brandon J. Shad conducted the search. James McKendry and Brandon J. Shad carried out the data analysis and prepared the figures. James McKendry, Leigh Breen, Brandon J. Shad and Carolyn A. Greig interpreted the results and drafted the manuscript.

Chapter 3: James McKendry, Carolyn A. Greig and Leigh Breen designed the study. James McKendry and Leigh Breen organized and carried out the experiments with the assistance of Boyang Liu and Shanat Baig. James McKendry, Sophie Joanisse, Gianni Parise and Leigh Breen performed the data analyses. James McKendry, Sophie Joanisse and Leigh Breen performed the statistical analysis of the data. James McKendry, Sophie Joanisse, Carolyn A. Greig and Leigh Breen interpreted the data and drafted the manuscript.

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1. Introduction

1.1. Challenges of Global Ageing

1.1.1. Ageing Population

The demographic of the UK population is rapidly shifting towards that of an ageing one (2015). Since 1960 the life expectancy of all countries in the EU has significantly increased and it is predicted that by 2060 average life expectancies of men and women will have increased to 84.7 and 89.1 years, respectively (2015). In the UK alone, the population is anticipated to increase by 25% between 2013-2060 from 64.1 million to 80.1 million (2015). The number of individuals over the age of 65 years is predicted to increase from 11.1 to 19.9 million, of which 7.6 million will be over the age of 80 years, signifying a 78.9% increase in the 'oldest old' in our society. Advancements in preventative and therapeutic medicine, lifestyle modifications have led to falling mortality rates and increasing longevity which, alongside declining fertility rates, have largely driven this demographic population shift (Harper, 2014). An ageing population presents a significant global socio-economic challenge to individuals and healthcare providers. However, the predicament is not that individuals are living longer; it is that individuals are spending an ever-expanding portion of their later years with a number of age-related diseases and poor quality of life (Seals et al., 2016).

1.1.2. Primary vs. Secondary Ageing

"Ageing is commonly characterized as a progressive, generalized impairment of function, resulting in an increasing vulnerability to environmental challenge and a growing risk of disease and death (Kirkwood, 2005)". The complexities of ageing can be broadly subdivided into two contributing categories, the first of which is primary/inherent

ageing, previously described by Holloszy as *"the inevitable deterioration of cellular structure and function independent of disease and environment"* (Holloszy, 2000). Primary ageing is largely determined by genetics. A large number of genes that contribute to the inherent ageing process and longevity have been identified, and are described in detail elsewhere (Sinclair and Guarente, 2006). Based on large-scale studies of identical twins, it has been estimated that genetic factors account for ~20-30% of lifespan (Hjelmborg et al., 2006, Finch and Tanzi, 1997, Herskind et al., 1996, Ljungquist et al., 1998). The remaining determinants of lifespan are disease factors and environmental influences (Holloszy, 2000); referred to as secondary or biological ageing. Secondary ageing is predominantly influenced by factors including, exercise, nutrition, smoking and ultraviolet radiation (Holloszy, 2000). These environmental factors, most notably physical activity and exercise, can impact the likelihood of progressing into older age with/without disease and physical impairments (Booth et al., 2012).

1.1.3. Lifespan vs. Healthspan

Originally, James Fries (1980) posited that geriatric medicine should focus on compressing the period of time individuals spend with disease and disability (Fries, 1980). This would result in a reduced total time of incurred illness, much closer to a natural end of life (Seals et al., 2016), often expressed as compression of the 'area under the morbidity curve'. Until recently, the primary focus has been the extension of lifespan. The lifespan of individuals around the world has increased and is projected to continue increasing (Kontis et al., 2017). However, healthspan is distinct from lifespan in that it represents the period of time individuals are able to maintain good health, unburdened by disease and ailment (Seals et al., 2016). Minimising the gap between healthspan and lifespan is

challenging. Ageing is complex and proposed as the foremost risk factor for the development of a number of diseases and conditions. Cardiac pathologies, cancer, stroke, diabetes, and neurodegenerative conditions are amongst the most prevalent diseases of older age (Niccoli and Partridge, 2012). These common diseases associated with older age can severely impact functional independence and quality of life, which is reflected in the numbers of older individuals reliant on health services (Sousa et al., 2016). Therefore, the challenge facing healthcare professionals, and researchers, is to determine whether it is possible to extend the number of 'healthy years' that individuals experience by preventing, or slowing the progression of, these age-associated health decrements.

1.2. Musculoskeletal Ageing

Skeletal muscle makes up ~40% of total body mass and is critical for a variety of mechanical and metabolic functions; including locomotion, basal metabolism, energy storage and nutrient deposition (Frontera and Ochala, 2015). Skeletal muscle mass is an independent predictor of all-cause mortality (Srikanthan and Karlamangla, 2014), emphasising the significance of maintaining this tissue mass. In young, healthy adults consuming adequate dietary protein and not engaged in regular structured exercise, muscle mass remains relatively constant throughout adulthood until ~40-50 y, following which it declines at a rate of ~0.5-1% each year (Hughes et al., 2002). The trajectory at which muscle mass loss occurs in older age is highly variable. Specifically, muscle atrophy can be drastically hastened when individuals experience major alterations to activity status such as periods of disuse/immobilisation (Wall et al., 2016) or the development of diseases (Morley et al., 2006).

1.2.1. Sarcopenia: Definition, Diagnosis and Prevalence

The loss of skeletal muscle mass with ageing is termed 'sarcopenia', proposed by Rosenberg in 1989, which the literal translation in Greek refers to *'poverty of the flesh'* (Rosenberg, 1989). Since inception, the definition of sarcopenia has been repeatedly modified to accommodate the progressive complexities of the syndrome. Cruz-Jentoft and colleagues, of The European Working Group on Sarcopenia in Older People (EWGSOP), proposed that the appropriate definition incorporate low muscle mass, muscular strength and/or physical performance (Cruz-Jentoft et al., 2010). Resulting in the definition most commonly used today *"Sarcopenia is a syndrome characterised by progressive and generalised loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death"* (Cruz-Jentoft et al., 2010). In 2018, ten years following the original EWGSOP meeting, the working definition of sarcopenia has been revised to include (i) low muscle strength (ii) low muscle quantity or quality and (iii) low physical performance: where probable sarcopenia is identified by criterion 1, diagnosis is confirmed by the addition of criterion 2, and severe sarcopenia is indicated by the addition of criterion 3 (Cruz-Jentoft et al., 2018). To obtain an estimate of the number of individuals suffering from sarcopenia with any degree of accuracy is problematic, as the prevalence of sarcopenia is highly dependent on the specific diagnostic tool that is used (Beaudart et al., 2015). Previous reports suggest that sarcopenia prevalence increases with age (Janssen et al., 2002), with ~5-13% of individuals between 60-70 y and ~11-50% of individuals >80 y diagnosed with sarcopenia (Morley, 2008, von Haehling et al., 2010); with small differences between men and women, feasibly due to the disparity in absolute peak muscle mass at the onset of muscle mass decline in the 4^{th}-5^{th} decade of life (Janssen et al., 2002). Sarcopenia presents a significant health concern for individuals, in particular

due to the negative impact on physical and metabolic function, which are tightly linked to disease development and mortality (Kodama et al., 2009, Li et al., 2017). The designation of an International classification of Diseases (ICD-10) code to sarcopenia, in September 2016, represents a significant step towards recognising age-related muscle loss as an independent disease requiring healthcare intervention (Cao and Morley, 2016, Anker et al., 2016).

1.2.2. Effects of Ageing on Skeletal Muscle

Sarcopenia is a syndrome resulting from the interaction of numerous physiological and lifestyle factors (i.e. primary and secondary ageing) (Narici and Maffulli, 2010). Mechanistically, neuromuscular changes, hormonal and immunological alterations, alongside reductions in physical activity and changes to nutritional habits with advancing age, contribute to the development of sarcopenia, and the rate at which this disease progresses (Narici and Maffulli, 2010). Neuropathies including α-motor neuron death coupled with continuous cycles of denervation and re-innervation underpin a decrease in the number of motor units and, hence, an increase in size of surviving motor units as sprouting axons re-innervate denervated muscle fibres (Campbell et al., 1973). Mitochondrial dysfunction and heightened inflammatory status (elevated interlukin-6 (IL-6), Interlukin-1 (IL-1), tumour necrosis factor alpha (TNF-a)) contribute to sarcopenia through muscle cell apoptosis (Narici and Maffulli, 2010). In addition, age-related muscle fibre loss occurs through a reduction in mechanical loading of muscle contractile components (physical inactivity), modifications to the hormonal environment (i.e., reductions in diurnal testosterone, growth hormone, IGF-1, oestrogen and other

androgens) which manifest as a shift towards muscle catabolism, resulting in a net loss of muscle protein mass (Narici and Maffulli, 2010). Moreover, impairments in satellite cell activation and proliferation likely reduce skeletal muscle regenerative capacity over the life-course (Narici and Maffulli, 2010). Taken together, these underlying age-associated physiological alterations potentially have serious consequences for the preservation of muscle mass and functional capacity in older age.

1.2.2.1. Skeletal Muscle Morphology and Architecture

Architecture

Ageing deleteriously impacts skeletal muscle architectural organisation (i.e. the macroscopic arrangement of muscle fibres) (Narici et al., 2003). It is well established that muscle architecture is one of the principal determinants of muscle function; primarily force generation and contraction velocity (Lieber and Friden, 2000). The main architectural features that determine skeletal muscle function include muscle length, fibre/fascicle length, fibre pennation angle and physiological cross sectional area (PCSA) (Figure 1). Specifically, muscle length describes the linear distance between the origin and insertion of the muscle. Fibre/fascicle length describes the distance between the intersection composed of the superficial aponeurosis and fascicle and the intersection composed of the deep aponeurosis and the fascicle. Pennation angle was defined as the internal angle composed of the fascicle and deep aponeurosis. Muscle thickness describes the vertical line from the superficial aponeurosis to deep aponeurosis (Fukutani and Kurihara, 2015). Lastly, the PCSA describes muscle volume relative to the arrangement of muscle fibres/fascicles (i.e., Muscle volume/fascicle length) (McPhee et al., 2018). Skeletal muscle architecture is of particular importance as the force produced by the

muscle is proportional to PCSA, and the force decreases in proportion to the cosine of the fibre pennation angle (McPhee et al., 2018).

Previously, it has been shown that PCSA was reduced in older compared with young individuals (Narici et al., 2003). A reduction in muscle thickness, fascicle length and pennation angle has also been demonstrated in old (60-85 years) compared with young (20-39 years) individuals (Kubo et al., 2003). In addition, young (~24 years) had greater muscle thickness and pennation angle of the quadriceps than older (~68 years) individuals, and muscle thickness was reported to be closely associated with maximal voluntary contraction (MVC) torque (Strasser et al., 2013). Clearly, alterations to skeletal muscle architecture with advancing age are relatively well characterised and may, in part, explain some of the functional decrements commonly observed in older individuals.

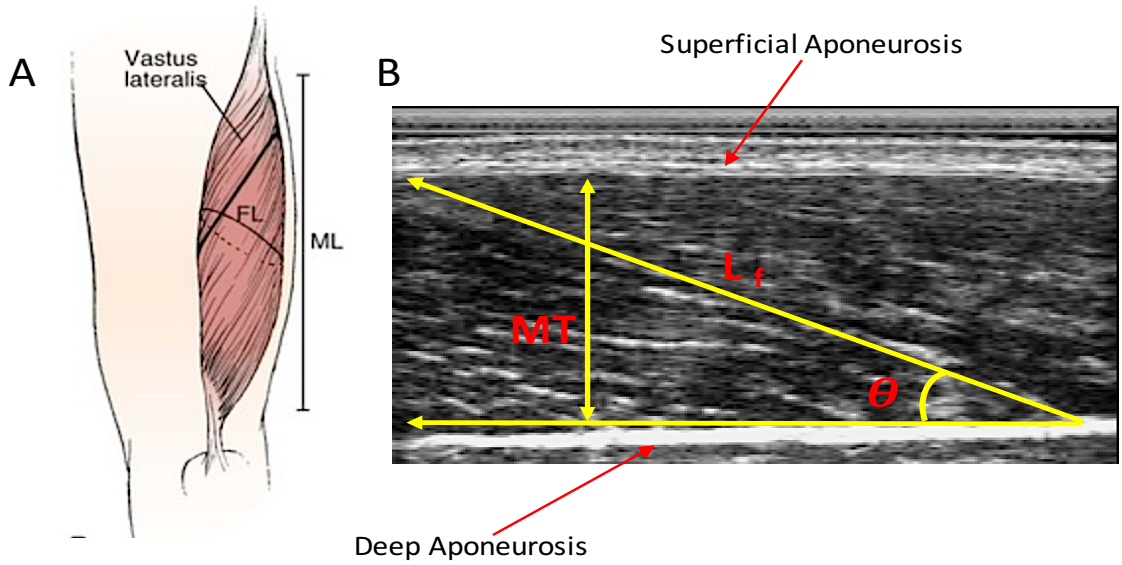

*Figure 1. The main architectural features shown in situ (A) and as an ultrasound image (B) that determine skeletal muscle function include muscle length, fibre/fascicle length, fibre pennation angle and physiological cross sectional area (PCSA, Indicated by the dotted circler on diagram A). L_f and FL, Fibre/Fascicle length; ML, Muscle length; MT, Muscle thickness; **θ**, Pennation angle.*

Muscle Cross-Sectional Area

The advancement of imaging techniques has enabled investigation into myocellular alterations and skeletal muscle morphology (i.e., muscle fibre cross sectional area (CSA) and fibre type distribution). A frequently observed characteristic of ageing muscle is the reduction in muscle CSA between 50 and 80 y (Goodpaster et al., 2006); driven by a decline in the size of individual muscle fibres (Lexell, 1995) and a reduction in fibre number (Lexell et al., 1988). It has been consistently observed that predominantly myosin heavy chain (MHC) II fibre size is reduced with advancing age. In an early cadaveric study, Lexell and colleagues discovered that MHC II fibre CSA was ~26% smaller in 80 y old individuals when compared with that of a 20 y old, whereas the reduction in MHC I fibre CSA was not as readily apparent (Lexell, 1995). Later work suggested that reductions in muscle CSA with ageing are predominantly driven by a significant reduction in the size of the Type II muscle fibres (Nilwik et al., 2013). However, the larger muscles and fibre CSA in these individuals may preclude the identification of fibre number losses. More recently, in a study that set out to determine the contributors to age-related muscle weakness and voluntary activation, it was shown that reductions in muscle mass and CSA are the result of equal contributions of fibre loss and atrophy (McPhee et al., 2018). Taken together, it is apparent that reductions in muscle fibre CSA and fibre number are a commonly observed hallmark of sarcopenia (Lexell et al., 1986) and may play a role in the loss of skeletal muscle function with ageing.

Satellite Cells

Skeletal muscle stem cells, termed ‘satellite cells’, are proposed to play a key role in regeneration and repair processes (Snijders et al., 2015). Located between the sarcolemma and basal lamina, satellite cells remain in a quiescent state until stimulation, upon which the cells are activated, proliferate and differentiate (Snijders et al., 2015). Differentiation allows satellite cells to form new myofibres, which can repair or replace damaged fibres, or fuse to existing myofibres to donate their nucleus and enable hypertrophy. Satellite cell activity and progression through the cell cycle is regulated by a number of transcription factors (i.e., Pax7) and myogenic regulatory factors (Myf5, MyoD, MRF4, and Myogenin). Additional factors including; hepatocyte growth factor (HGF), myostatin (Mstn), Notch/Delta1, interleukin-6 (IL-6), mechano-growth factor (MGF), and insulin like growth factor-1 (IGF1) contribute to the regulation of satellite cells during differing stages of their myogenic program (Snijders et al., 2015). Ageing appears to exert a deleterious impact on skeletal muscle satellite cell number, predominantly in Type II muscle fibres (Verdijk et al., 2014). Satellite cells have been shown to decrease between 25-70% in the *vastus lateralis*, *biceps brachii* and *tibialis anterior* in old vs. young individuals. However, studies have also reported no change in the satellite cell pool with ageing (Roth et al., 2000, Dreyer et al., 2006). Much of the discrepancy between studies could be the result of a scarcity of fibre-type specific satellite cell data (Verdijk et al., 2007). A reduction in the intramuscular satellite cell pool and/or impairments in satellite cell activation and proliferation in response to exercise stimuli, may adversely affect muscle remodelling and long-term maintenance (Snijders et al., 2009). Others have suggested that a reduction in the satellite cell pool in aged skeletal muscle may not be limiting for skeletal muscle remodelling, and that diminution of the satellite cell pool is unlikely to prevent repair of aged skeletal muscle following damage as a result of chronic

exercise training (Thornell et al., 2003). Therefore, the role satellite cells perform in skeletal muscle repair and remodelling with ageing is somewhat contentious. Further investigation is necessary to fully characterise the influence of ageing on satellite cells content and responsiveness, and whether chronic exercise training, over many years, is a viable strategy to preserve satellite cell integrity. Such investigations could ultimately yield new treatment methods for sarcopenia.

Myonuclei

Skeletal muscle fibres are one of only a few multinucleated cells in the human body. Each myonucleus is responsible for the gene transcription and protein synthetic activity of a finite region of skeletal muscle cytoplasm; termed the myonuclear domain (Cheek, 1985). It is plausible to consider that increases, or decreases, in skeletal muscle CSA in response to chronic exercise or ageing would be mirrored by alterations to the total number of myonuclei. Upon stimulation, satellite cells eventually form new myonuclei or return to a state of quiescence (Verdijk et al., 2014). Similar to satellite cells, the role of the myonuclear domain in skeletal muscle maintenance and adaptation is debatable. It has been shown that myonuclear domain is decreased in older adults (50-69 years) compared with younger adults (18-49 years), expressly in Type II muscle fibres (Verdijk et al., 2014). Single fibre analysis of immobilized rodent muscle (i.e. a model of accelerated ageing), suggests that myonuclei are not lost, but that the domain size does decrease with fibre atrophy (Wada et al., 2002). Regardless, the dynamic between satellite cells and myonuclei in the maintenance and repair of skeletal muscle is poorly understood, and it

remains to be determined whether satellite cells and myonuclear domain are integral to the maintenance of muscle mass and function with advancing age.

Capillarisation

Sufficient delivery of oxygen and nutrients to skeletal muscle is critical to increase and maintain skeletal muscle mass and function. Therefore, adequate muscle perfusion from the surrounding capillary network is vital. Previously, it has been shown that ageing adversely impacts skeletal muscle microvasculature (Parizkova et al., 1971, Groen et al., 2014) and total limb blood flow (Donato et al., 2006, Skilton et al., 2005). This is clearly demonstrated by substantial alterations to the capillary network, with reported reductions in (i) capillary contacts per myofibre, (ii) individual capillary-to-fibre ratio (C:Fi) and (iii) capillary fibre perimeter exchange (CFPE) index (Hepple et al., 1997b). Conversely, exercise training, specifically endurance type, elicits significant adaptations to the skeletal muscle capillary network. Previously, it has been shown that 8 weeks of cycle training led to a 20% increase in capillary density in young men (Andersen and Henriksson, 1977). Furthermore, older individuals can also increase skeletal muscle capillarisation in response to endurance training (Gavin et al., 2007). The difference in basal capillarisation between young and old reflects an age-related decline, which can potentially be offset as both groups demonstrate the same capacity to increase capillarisation following endurance exercise training (~20%) (Gavin et al., 2007). Reductions in capillarisation with ageing may contribute to an accelerated loss of muscle mass and insulin resistance, due to poor delivery of nutrients (i.e., amino acids, glucose, insulin), which may have significant consequences for an individual's functional capacity and metabolic health. Importantly,

impairments in this skeletal muscle perfusion may be overcome with exercise training, the extent to which this is possible with chronic endurance training, over many years, warrants further investigation.

1.2.2.2. Neuromuscular Properties

Ageing skeletal muscle characteristically undergoes neuromuscular alterations. In ageing muscle, muscle fibre denervation occurs, predominantly in Type II fibres, due to a progressive loss of motor neurons (Degens, 2007). Previous studies in cadavers have revealed that individuals aged 75 y have ~30% fewer motor neurons supplying the muscles of the lower limbs (Tomlinson and Irving, 1977). In some cases, the neural drive to denervated fibres can be salvaged through re-innervation by sprouting axons of nearby motor neurons. This leads to an increased size of the surviving motor units, which may have significant consequences for muscle function; as larger motor units require much greater stimulation for force generation. In addition to this, ageing skeletal muscle undergoes extensive neuromuscular remodelling, specifically older individuals (~71 years) demonstrate ~44% fewer motor units (assessed by motor unit number estimates (MUNE)), less stable neuromuscular junction transmission and slower discharge rates when compared with young individuals (~25 years) (Piasecki et al., 2016c). When coupled with the reduction in muscle CSA, this had notable consequences for motor function as shown by a reduction in MVC by ~32% when compared with younger individuals.

1.2.3. Consequences for Function and Health

Physiological function and performance typically deteriorates with advancing age, particularly maximal aerobic capacity and muscular strength. The cardiovascular and respiratory systems, which play a critical role in delivering oxygen and nutrients to the rest of the body whilst concurrently removing metabolic waste products, are adversely affected by ageing (Harridge and Lazarus, 2017). Maximal aerobic capacity (VO_{2max}), a product of both cardiac output (Q) and maximal arterio-venous difference (A-VO_{2diff}), decreases at a rate of ~1% per year after the age of 25 years in untrained individuals (Astrand et al., 1973, Buskirk and Hodgson, 1987). Young healthy men and women aged 20-29 y typically display a VO_{2max} of 54.4 and 43.0 $ml.kg^{-1}.min^{-1}$, respectively (Loe et al., 2013). By age 60-69 y, VO_{2max} is significantly reduced to 39.2 and 31.1 $ml.kg^{-1}.min^{-1}$ for men and women, respectively (Loe et al., 2013). The reduction in VO_{2max} with advancing age is predominantly caused by a decline in cardiac output (Lambert and Evans, 2005), impairments in skeletal muscle oxidative capacity (Betik and Hepple, 2008), a reduction in fat-free mass and increased fat mass (Kuk et al., 2009); all of which are typically exacerbated with advancing age. Due to a close association of VO_{2max} with morbidity and mortality, the development and maintenance of high levels of aerobic fitness should be a primary consideration for healthy ageing.

Ageing exerts a significant influence on the musculoskeletal system, with reductions in muscular strength and power. Muscular strength declines at a rate of ~3-4% and 2.5-3% annually in men and women, respectively (Manini and Clark, 2012). Similar declines in muscular power have been observed (Izquierdo et al., 1999), with dire consequences for functional capacity in older individuals. Specifically, a reduced ability to carry out basic functional tasks required for independent living such as ambulation, rising from a

chair/bath, or opening a jar (Skelton et al., 1994). Previously, it has been shown that individuals over 60 y in the lowest tertile of strength(i.e., the weakest old), were almost 50% more likely to die due to all-cause mortality when compared with the upper tertile (i.e., the strongest old) (Ruiz et al., 2008), clearly demonstrating the importance of maintaining muscle strength and power in older age. The underlying mechanisms responsible for the decline in muscular strength observed with ageing are contentious. There are two key contributory elements that have been suggested to drive the reduction in muscular strength and power; neurological and musculoskeletal factors (Clark and Manini, 2010). Neurological alterations include the reduction of motor unit recruitment and α-motoneuron discharge rate alongside neuromuscular junction transmission failure (Clark and Manini, 2010). Conversely, the muscular factors that contribute to the reduction in voluntary force output are alterations to the muscle contractile machinery (i.e., excitation-contraction uncoupling, fibre-type shifts and infiltrations) and the decline in fat-free mass due to fibre atrophy (Clark and Manini, 2010). Although there is still some debate of the contribution of neural and muscular factors to sarcopenia, it has recently been demonstrated that reductions in muscle mass appear to be the dominant driving force behind strength reductions with advancing age (McPhee et al., 2018).

Greater levels of physical function are associated with lower risk of all-cause mortality (Kodama et al., 2009, Blair et al., 1989). Aerobic capacity and muscular strength are examples of bodily functions that require the successful integration of the cardiovascular, respiratory and neuromuscular systems. As a result, targeted strategies to combat declines in physiological function are imperative to extend the healthspan, and improve the quality of later life for individuals. It is well established that endurance exercise training improves endurance performance, mitochondrial function, capillarisation, glucose/insulin

metabolism and drastically reduces cardiovascular risk profile (Egan and Zierath, 2013). Resistance exercise has a more substantial effect on the development/maintenance of muscular strength and power, basal metabolic rate and locomotor activities (Egan and Zierath, 2013). As a result, a combination of the two types of exercise in promoting a 'healthy lifestyle' has been reflected in physical activity guidelines for older adults (Piercy et al., 2018). Unfortunately, a significant number of older individuals fail to meet the current recommended physical activity guidelines (Jefferis et al., 2014). The importance of skeletal muscle function to an individual's ability to age 'healthily' and independently, necessitates continued research into the mechanisms underlying age-related physiological deterioration in aerobic capacity and muscle strength and to develop strategies that offset, and perhaps prevent, this deterioration.

1.3. Regulation of Muscle Plasticity

Skeletal muscle is a highly plastic tissue, capable of momentous change in response to the environmental demands placed upon it. This is best illustrated in extreme situations, for example, following 6 months of spaceflight skeletal muscle volume is reduced by ~4-20% in the lower limbs despite counter-measures focussed on skeletal muscle maintenance (Gopalakrishnan et al., 2010). Conversely, chronic resistance training elicits substantial skeletal muscle hypertrophy (i.e., ~7-10% following 10 weeks of resistance training (Damas et al., 2018). A reduction in the body's most abundant tissue, skeletal muscle, unavoidably leads to an increased risk for disability and disease (Visser et al., 2005). Exercise and nutrition are key determinants in the regulation of skeletal muscle, and may play an important role in mitigating the negative effects of ageing. The change in skeletal muscle tissue abundance is brought about by changes in the synthesis and degradation of

muscle proteins, tightly controlled by a cascade of intracellular signalling proteins (Figure 2) and satellite cells which alter protein expression and drive alterations in skeletal muscle phenotype. Much of what has been learned so far is the result of the development and integration of sophisticated analytical tools to investigate the mechanistic control of skeletal muscle. It is essential to continue to progress understanding of the complexities of ageing and how it impacts musculoskeletal health.

1.3.1. Skeletal Muscle Protein Turnover

Skeletal muscle mass is regulated by diurnal oscillations in muscle protein synthesis (MPS) and muscle protein breakdown (MPB), both of which are dynamic in nature and act to constantly turn over skeletal muscle protein mass (Kim et al., 2016a). These processes are radically influenced by ageing, physical activity and nutrition. The balance between these two opposing processes determines whether muscle protein mass is gained or lost. In young healthy adults consuming adequate dietary protein, MPS and MPB are in net protein balance (NPB), thus maintaining skeletal muscle mass (Phillips et al., 1997). MPS occurs at a rate of ~1-2% per day (Carraro et al., 1990), such that the entirety of skeletal muscle an individual has will have undergone complete renewal every ~3-4 months. It is thought that the primary role of MPB is to maintain adequate concentrations of intramuscular amino acids for the synthesis of new proteins and the removal of dysfunctional/damaged proteins (Tipton et al., 2018). However, due to methodological difficulties inaccurately measuring rates of MPB (i.e., contributions of amino acids from non-muscle tissues, catheterization of an artery/deep vein, and the necessity of physiological steady state), little is known about the role of proteolysis in overall NPB (Tipton et al., 2018). It is generally accepted that changes in skeletal muscle mass are primarily driven by alterations in MPS, with MPB playing a lesser (but potentially

important) role (Phillips et al., 1997). In the postabsorptive state, rates of MPB exceed MPS resulting in an overall negative NPB which results in the gradual loss of muscle protein mass (i.e. catabolism). Specifically, young men and women (~23 years) during the postabsorptive state demonstrate MPS rates of ~0.06 $\%.h^{-1}$ whereas MPB rates were estimated to be ~0.12 $\%.h^{-1}$ (Phillips et al., 1997). Furthermore, when exposed to a resistance exercise stimulus, both MPS and MPB increased simultaneously; though overall NPB was less negative due to a greater relative rise in MPS (Phillips et al., 1997). However, the intake of dietary protein, in particular the amino acid leucine, stimulates a robust increase in MPS (Biolo et al., 1995) and is critical for the generating a positive NPB (i.e. anabolism). Indeed, the stimulation of MPS is synergistically enhanced by combined protein nutrition and resistance exercise stimuli (Pennings et al., 2011) and this response is influenced by protein dose (Moore et al., 2009a), Source (Tang et al., 2009), Quality (Churchward-Venne et al., 2014) and timing (Areta et al., 2013). Thus, repeated stimulation of this anabolic response (i.e. high protein diet and frequent resistance exercise) leads to an increase in skeletal muscle mass over time (Morton et al., 2018, Hoffman et al., 2009).

1.3.2. Translational Regulation of Muscle Protein Turnover

Central to the regulation of MPS and skeletal protein mass, is the highly conserved serine/threonine kinase, the mammalian target of rapamycin (mTOR). In humans, this protein complex is present in two forms, mTORC1 and mTORC2 both of which consist of a number of protein sub-units (Laplante and Sabatini, 2012). Primarily mTORC1 regulates a number of growth and metabolic pathways, whereas mTORC2 is largely responsible for cell proliferation and survival (Laplante and Sabatini, 2012). Both complexes are large, and contain some similar protein sub-units (mTOR, mLST8, DEPTOR and the Tti1/Tel2

complex). However, the proteins differ structurally in that mTORC1 contains Raptor and PRAS40, and mTORC2 contains Rictor, mSin1 and Protor1/2. This thesis will focus on mTORC1, as the upstream and downstream targets are the better characterised of the two complexes with mTORC1 implicated in age-related skeletal muscle deterioration. mTORC1 integrates a number of critical upstream signals, including mechanical stress, growth factors, amino acids, energy status and oxygen levels and controls several downstream pathways with divergent cellular processes including macromolecule synthesis, autophagy, cell cycle, growth and metabolism (Laplante and Sabatini, 2012). This array of biological functions has led to mTOR frequently being referred to as a 'master regulator' of cell plasticity (Hsieh, 2012).

The upstream regulation of mTORC1 has been the topic of intense study, although regulatory factors remain to be fully elucidated. First the heterodimer tuberous sclerosis 1 (TSC1) and tuberous sclerosis 2 (TSC2), serves to activate the GTPase-activating protein (GAP) Ras homolog enriched in brain (Rheb). GTPase has been shown to modulate mTORC1 activity (Laplante and Sabatini, 2012). The GTP-bound form of Rheb strongly activates mTORC1 kinase activity. TCS1/2 negatively regulates mTORC1 by converting Rheb to its GDP bound state, this regulator of mTORC1 activity integrates signals from a number of upstream stimuli (i.e., growth factors/insulin) (Laplante and Sabatini, 2012). Protein kinase B (akt/PKB) also acts directly by invoking dissociation of PRAS40 from mTORC1 (Laplante and Sabatini, 2012); stimulating its kinase activity. Amino acids activate mTORC1 activity through a TSC2 independent mechanism involving Rag-GTPases. Conversely, Adenosine monophosphate-activated protein kinase (AMPK) acts as a negative regulator of mTORC1 activity, both directly and through activation of TSC2 (Laplante and Sabatini, 2012). Finally, mitogen-activated protein kinase (MAP4k3),

mammalian vacuolar protein sorting 34 homolog (hVPS34), inositol polyphosphate monokinase (IPMK) and phosphatidic acid (PA) have all been implicated in mTORC1 activity.

Downstream of mTORC1 lie a number of relatively well characterised key cellular processes, responsible for maintenance and growth of skeletal muscle mass (Laplante and Sabatini, 2012). mTORC1 is thought to regulate MPS via two primary mechanisms. First, the phosphorylation and inhibition of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), which prevents association with cap dependent protein eIF4E and enables formation of the eIF4F complex, thereby promoting cap dependent translation. Second, the phosphorylation and activation of ribosomal protein S6 kinase (p70S6K), which upregulates ribosomal biogenesis, mRNA translation initiation and elongation for MPS (Laplante and Sabatini, 2012). Thus, mTORC1-mediated signalling events play a critical role in the regulation of MPS (Wackerhage and Ratkevicius, 2008). A simplified schematic detailing the intracellular signalling mechanisms controlling skeletal muscle protein synthesis are illustrated in Figure 2.

The translational regulation of MPS in response to exercise and nutritional stimuli is an important focus for integrative physiology researchers. It is typically demonstrated that mTORC1 and its downstream targets (i.e., 4E-BP1 and p70S6K) are rapidly phosphorylated and remains so for hours following, resistance, exercise (Burd et al., 2010a, Mitchell et al., 2014). The activation of these proteins and their upstream signalling proteins are included in the analysis of acute exercise studies to gain mechanistic insight into the regulatory control of MPS in response to exercise. The impact that ageing exerts on these processes is discussed later in this chapter.

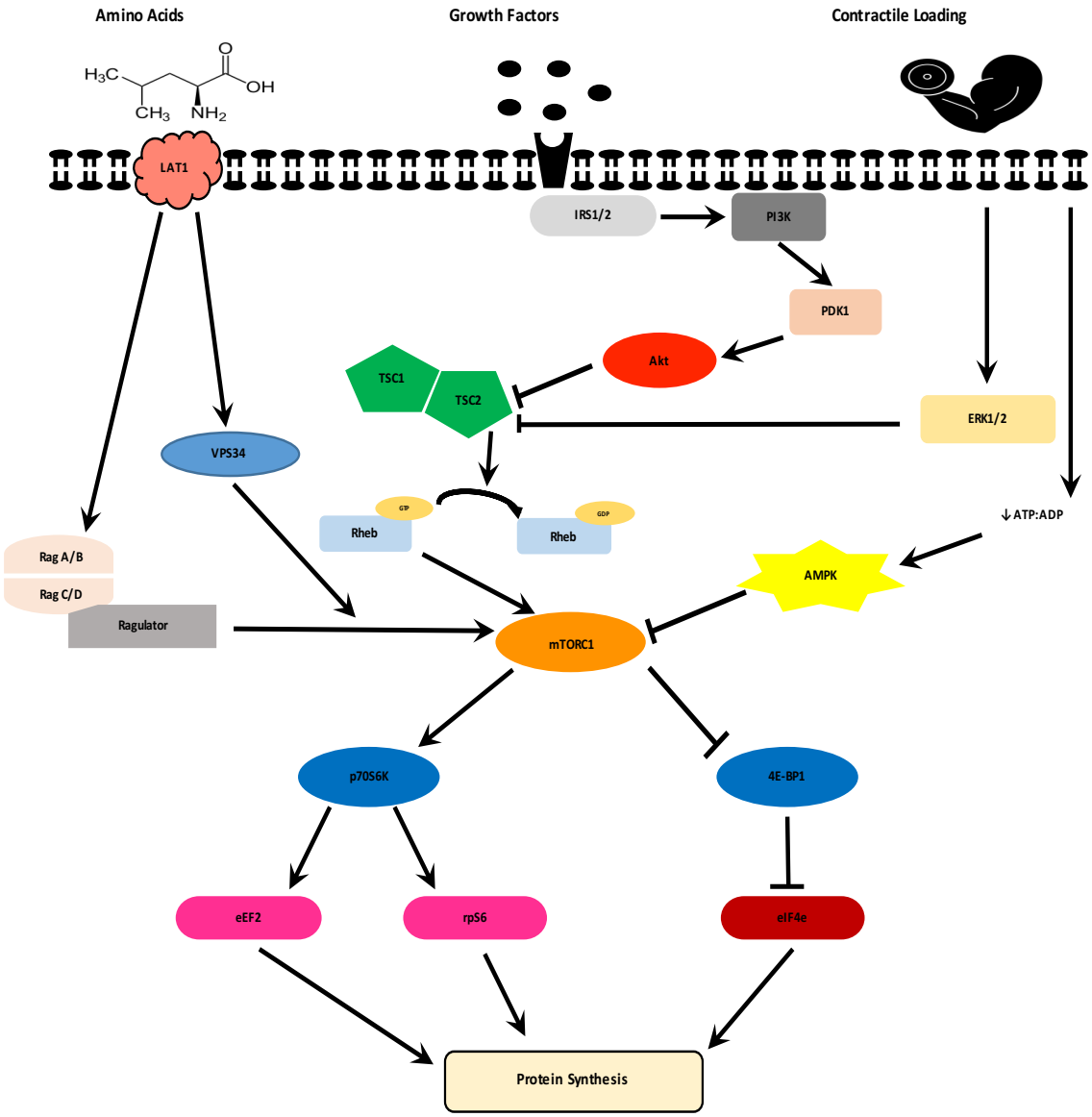

Figure 2. *A simplified schematic representation of the intracellular signalling mechanisms controlling skeletal muscle protein synthesis*

1.3.3. Exercise, Nutrition and Muscle Anabolism

Exercise, in particular resistance exercise, significantly modulates muscle (myofibrillar) protein turnover. The complexity involved in measuring MPS and MPB during an exercise bout means very few studies have attempted to do so (Carraro et al., 1990). As a result, most studies have investigated the effect of exercise on MPS and MPB in the post-exercise recovery period (early- and late-phase). It has been consistently shown that resistance exercise is a potent stimulus for elevating MPS following exercise (Burd et al., 2010b). Typically, during acute substrate-specific stable isotope infusion trials, exercise increases MPS by ~100-220% over several hours post-exercise (Phillips et al., 1997, Moore et al., 2009b), which remain elevated above basal rates for up to 48 h (Phillips et al., 1997). Exercise and feeding appear to work synergistically to maximally stimulate MPS and promote skeletal muscle remodelling (Moore et al., 2009b, Dreyer et al., 2008, Witard et al., 2014). The extent to which MPS is stimulated, and duration of this anabolic response, can be modulated by altering the training variables of the resistance exercise bout (i.e., load (Burd et al., 2010b), volume (Burd et al., 2010a), rest-interval (McKendry et al., 2016)) and is the subject of intense study.

Frequent stimulation of MPS and net protein accretion through resistance exercise loading and amino acid provision, is thought to underpin increases in muscle mass (Phillips, 2004). However, there appears to be a large degree of incongruence between the acute increases in MPS, markers of anabolic signalling and the degree of muscular hypertrophy and strength development (Hubal et al., 2005). For example, Baar and Esser have shown, in rodents, that upregulation of signalling 6 hours following an acute bout of exercise is a good marker of muscle mass gain over time (Baar and Esser, 1999). Despite the lack of sufficient human data it has become all too common to extrapolate findings

from acute investigations of MPS as predictive of chronic changes in muscle mass (Murton and Greenhaff, 2013). The acute rise in MPS following a bout of resistance exercise was not related to the subsequent increase in muscle mass over a 16 week training period (Mitchell et al., 2014). The authors postulated that the lack of relationship between acute MPS and chronic muscle hypertrophy could be due to a number of factors, namely; the specificity of the muscle protein fraction measured (i.e., myofibrillar vs non-myofibrillar), the inclusion of measures at later time points (i.e. >4 h post-exercise), or alterations to MPB throughout the training programme (Mitchell et al., 2014). It has recently been shown that elevated MPS at the beginning of a training programme are directed towards the repair of damaged muscle protein, with the continuation of the exercise stimulus for 10 weeks leading to a greater proportion of MPS being directed towards muscular hypertrophy (Damas et al., 2016). Nevertheless, the lack of a clear relationship precludes the use of acute MPS as a predictive tool for quantitative estimates in responsiveness to a resistance exercise training programme. Alternatively, it is apparent that stable isotopic tracer approaches such as D_2O, that permit measurement of muscle protein turnover over longer periods, offer a logical step to characterise an individual's muscle hypertrophic/remodelling responsiveness.

The introduction of stable isotopic tracer methodologies has enabled exercise physiologists to study the complex mechanisms that govern muscle mass regulation (for comprehensive resources on stable isotopic tracer methodology see (Wolfe and Chinkes, 2005) (Kim et al., 2016a) and (Wilkinson, 2018)). Administered orally, D_2O rapidly equilibrates in body water and is incorporated into multiple metabolic pathways (lipid, glycogen, protein and RNA/DNA synthesis). D_2O confers many benefits when compared with traditional tracer methodologies (Brook et al., 2017). The ease of administration and

maintenance of steady-state make measurements of bound product and precursor-labelling in saliva, over periods of hours-days-weeks-months (i.e., integrated MPS (iMPS)) feasible. In addition, study participants are able to continue their habitual daily activity, which enables the investigation of an individual's muscle protein turnover in a 'free-living' environment without the confounding influence of the restrictive laboratory setting. Whereas, conducting an acute isotope infusion investigation within a strictly controlled laboratory setting is problematic when attempting to translate the study findings to chronic changes in muscle mass under free-living conditions (Mitchell et al., 2013). The advantages previously highlighted make D2O an ideal candidate to study human muscle protein metabolism.

Chronic exercise training and individual training status, influences the MPS response to exercise. Previously, it has been shown that 8 weeks of unilateral resistance exercise alters the temporal pattern of MPS to acute exercise in young individuals (Tang et al., 2008). In the untrained leg, mixed muscle FSR was elevated at 4 h post-exercise, and remained elevated above baseline for 28 h post exercise. In the trained leg, the amplitude of the mixed muscle MPS response was greater, and the duration of the MPS response shorter, compared with the untrained leg, returning to basal values by 28 h post-exercise. However, no change in myofibrillar MPS was evident following 8 weeks of leg resistance exercise training in another study (Kim et al., 2005). The apparent difference between these studies is likely the specific protein fractions measured in which MPS was measured (i.e., mixed vs. myofibrillar protein). Alterations to acute MPS responsiveness are not limited to temporal changes, Wilkinson et al. (2008) demonstrated that the acute MPS response becomes exercise mode-specific following a period of endurance or resistance training, such that resistance training elicited an increase in the synthesis of myofibrillar

proteins (i.e. contractile) whereas endurance exercise stimulated an increase in mitochondrial protein synthesis (Wilkinson et al., 2008b). The individuals recruited in the studies previously highlighted were considered 'trained' following ~8-16 weeks of exercise training; whether or not the observed alterations to acute MPS would hold true in individuals who had been training for many years is unknown.

Exercise is an effective treatment for the unfavourable shifts in body composition that occur with ageing. Both exercise modalities, resistance and endurance, have been shown to reduce visceral and subcutaneous body fat by ~4-10% with 16-32 weeks of training (Slentz et al., 2004, Ibanez et al., 2005). Additionally, it has been repeatedly shown that chronic resistance exercise increases skeletal muscle mass in both young (Mitchell et al., 2012a, West and Phillips, 2012) and older individuals (Peterson et al., 2011). Thus, it has been effectively shown that both resistance and endurance exercise modalities may be effective tools to help slow the deterioration of body composition with advancing age (Harber et al., 2012, Harber et al., 2009, Peterson et al., 2011). As outlined earlier, the importance of increasing, or preserving, skeletal muscle mass is crucial for maintenance of physical function, independence and quality of life during later years. Thus, studies focussed on exercise interventions to maintain skeletal muscle mass, and the investigation of mechanisms responsible for its regulation, are vital.

Exercise training induces significant alterations to skeletal muscle morphology. Changes in skeletal muscle morphology underpin the augmentation of muscle mass and subsequently physical function (Snijders et al., 2009), which is often compromised in older individuals. In young individuals, in response to chronic exercise training, studies have repeatedly demonstrated changes in architectural arrangement of skeletal muscle fibres (Brook et al., 2015, Kim et al., 2015) and muscle fibre hypertrophy (in particular

Type II) (McCall et al., 1996, Mitchell et al., 2013, Staron et al., 1990, Staron et al., 1994). Moreover, it has been shown in a number of studies that in response to an acute exercise stimulus, skeletal muscle robustly upregulates satellite cell number in the subsequent days (McKay et al., 2009, Snijders et al., 2014). Thus, when exposed to a chronic resistance exercise stimulus, it is thought that the satellite cell pool undergoes notable expansion (Kadi et al., 2005). However, the effect of exercise on other muscle morphological features is less clear. Studies investigating changes in skeletal muscle capillarisation (Hepple et al., 1997b, Hagerman et al., 2000) and myonuclei (Verdijk et al., 2014, Snijders et al., 2016) have yielded conflicting findings. Significant increases in myonuclei content have been reported (Verdijk et al., 2014) in response to exercise training whereas other studies have demonstrated no changes to the myonuclear content of human skeletal muscle (Snijders et al., 2016). This may indicate that with chronic exercise training, the age-associated changes to skeletal muscle morphological features (i.e., reductions in fibre CSA, capillarisation, satellite cell no., myonuclei) may be preventable, however very few chronic training studies have been undertaken making this difficult to reconcile.

For a long time, it was assumed that older individuals were unable to adapt to an exercise stimulus. This appears not to be the case, and with exercise training it has been shown that older individuals can successfully improve physical function (i.e., aerobic capacity and muscular strength) (Coggan et al., 1992, Schoenfeld et al., 2015). However, studies investigating whether exercise training in older adults can facilitate shifts in body composition and facilitate skeletal muscle morphological alterations yield conflicting findings. Previously, exercise training in older individuals has demonstrated that improvements in body composition are achievable (i.e., lower body fat and greater muscle mass) (Willis et al., 2012). Whereas, others have shown that with 6 weeks of resistance

training older individuals failed to increase muscle mass when compared with young (Brook et al., 2016), suggestive of a blunting to exercise adaptation. Therefore, with advancing age the idea that older individuals retain the capacity to adapt to exercise similarly to young individuals (Harber et al., 2012) remains contentious and warrants further investigation.

1.3.4. Dysregulated Muscle Protein Turnover in Ageing

An impairment in the capacity to upregulate MPS in response to anabolic stimuli in older individuals has been termed age-related muscle 'anabolic resistance' and is considered a primary contributor to the loss of skeletal muscle mass with advancing age. Early studies investigating differences in protein turnover between young and older adults concluded that reductions in the basal rates of MPS were the fundamental drivers of age related loss of muscle mass (Yarasheski et al., 1993, Welle et al., 1993). In addition, it has been suggested that elevations in basal MPB contribute to the loss of muscle protein in older individuals (Trappe et al., 2004). However, if reductions in basal MPS and elevations in basal MPB were responsible for muscle protein loss, it would likely occur at a much greater rate than is characteristically observed in older individuals (~1-1.5% per decade) (Hughes et al., 2001). It has been demonstrated that differences in the basal rates of MPS were imperceptible between younger and older individuals (Volpi et al., 2001), however the inability to upregulate MPS in response to the ingestion of essential amino acids (EAA) (Cuthbertson et al., 2004) and resistance exercise (Kumar et al., 2009) was evident in old compared with young individuals. This finding has been supported by data from a number of subsequent studies, and summarised in a recent systematic review (Shad et al., 2016). Conversely, a number of studies have shown no evidence of anabolic resistance in response to protein feeding and resistance exercise (Koopman et al., 2009,

Paddon-Jones et al., 2004, Symons et al., 2011). The discrepancies observed between those that identified anabolic resistance and those that did not, are most likely the result of experimental methodology differences used to investigate the MPS response. For example, i) manipulating exercise volume ii) the dose/source/matrix of protein provided iii) the specific subfractions of muscle protein measured iv) time frame of the MPS assessment as previously highlighted (Shad et al., 2016). Regardless, it is evident that throughout the ageing process a multitude of alterations occurs at various stages, impairing the ability of older muscle to upregulate MPS in response to typically anabolic stimuli and ultimately driving the progression of sarcopenia. Conceivably, the amount of habitual physical activity may also play a role in modifying the responsiveness of older muscles. Thus, it is imperative that investigations to delineate the relative contribution of ageing and increasing physical inactivity to the observed anabolic resistance, are pursued.

1.3.5. Mechanisms of Age-related Muscle Anabolic Resistance

The mechanisms regulating the impaired responsiveness of older muscle to anabolic stimuli (i.e., protein feeding and exercise) remain to be fully elucidated. Multiple theses for the development of anabolic resistance with ageing have been proposed (i.e., dysregulation of amino acid availability, delivery and perfusion, inflammation, alterations to anabolic signalling and reductions in physical activity) (Burd et al., 2013, Breen and Phillips, 2011). The complex mechanisms underpinning anabolic resistance is illustrated in Figure 3.

1.3.5.1. Protein Digestion, Absorption and Muscle Perfusion

Protein digestion and absorption kinetics are modified with advancing age (Fujita and Volpi, 2004). Reduced protein (amino acid) availability may have significant consequences for skeletal muscle remodelling. Manipulating the dose, source and pattern of protein intake may be strategies employed to mitigate these impairments. It has been demonstrated that older individuals require significantly larger doses of protein in order to achieve the same degree of stimulation of MPS compared with young adults (Moore et al., 2014). Perhaps a result of a greater degree of splanchnic extraction of the amino acid leucine in older individuals (Boirie et al., 1997). This would lead to a lower systemic availability of leucine, and likely other amino acids, for the upregulation of MPS and skeletal muscle remodelling. In addition, a reduction in skeletal muscle perfusion may be a significant contributor to the purported insensitivity of older muscle to anabolic stimuli. Impaired delivery and perfusion, thus nutritive blood-flow, of skeletal muscle has also been observed in older individuals (Fujita et al., 2009, Meneilly et al., 1995, Rasmussen et al., 2006) and when blood flow is restored pharmacologically, rates of MPS are restored (Timmerman et al., 2010). Conversely, resistance exercise training can enhance leg muscle microvascular blood flow, however this did not result in an augmented MPS response in older men (Phillips et al., 2015), making it difficult to draw firm conclusions regarding the effect of muscle perfusion on MPS. Furthermore, an increase in EAA availability in older muscle was not sufficient to stimulate an increase in the expression of amino acid transporters, L amino acid transporter (LAT1)/solute-linked carrier (SLC)7A5 and system amino acid transporter (SNAT2)/ solute-linked carrier (SLC)38A2, beyond that of resistance exercise (Dickinson et al., 2013). Taken together, this suggests that older

individuals require a considerably greater anabolic stimulus in order to stimulate MPS to the same extent as younger individuals.

1.3.5.2. Inflammation and Obesity

It is generally accepted that body fat increases with advancing age, and ageing promotes the redistribution of fat to ectopic regions; both of which are a significant health concern (Ortega et al., 2016). Computed tomography (CT) and immunohistochemistry have shown that adipose tissue accumulates in older muscle (Goodpaster et al., 2000a, Smeuninx et al., 2017, Goodpaster et al., 2000b). Increases in intramyocellular lipid (IMCL) content, may play a role in the observed reduction in anabolic sensitivity (Rivas et al., 2012, Pan et al., 1997). Ostensibly, this may occur through the activation of pro-inflammatory cytokines (toll-like receptor 4, tumour necrosis factor alpha (TNFα), and nuclear factor kappa-light-chain enhancer of activated B cells (NFκB)) (Holland et al., 2011) and lipid-induced insulin resistance (Stephens et al., 2015). Non-steroidal anti-inflammatory drug (NSAID) have been shown to reduce the low grade inflammatory status experienced with ageing, postprandial MPS was significantly augmented compared to a control group (Rieu et al., 2009). It should be noted that this study was conducted in largely heterogeneous rats which may explain some inconsistent findings. Moreover, a mechanistic link between C-Reactive Protein (CRP), a well-established biomarker of systemic inflammation, and the decline in muscle mass has been established (Wåhlin-Larsson et al., 2017). Indicating that CRP may, in part, be responsible for deleterious changes with muscle mass and its regulation with advancing age. This effect may be mediated through activation of markers of stress and energy status (i.e., AMPK) and a reduction of in the phosphorylation of Akt and rpS6, both of which are implicated in

upregulating MPS in the mTORC1 Pathway. As a result, the prevention of ectopic fat accumulation and a reduction in the chronic low-grade inflammation with ageing should be a key concern. The continuation of exercise throughout life may act as a suitable countermeasure by promoting a healthy body composition.

1.3.5.3. Sex Hormones

Sex hormones play a major role in regulation MPS and MPB, and hormonal modifications are one of the many contributing factors towards the progression of sarcopenia (Narici and Maffulli, 2010). An optimal hormonal milieu provides the best systemic conditions in which to maintain skeletal muscle mass with advancing age. However, a decline in hormones responsible for skeletal muscle anabolism (i.e., growth hormone (GH), insulin-like Growth Factor 1 (IGF-1), testosterone along with other androgens) and an elevation in catabolic factors (i.e., IL-1, IL-6, TNF-α and myostatin) shifts the stimulus towards catabolism (Narici and Maffulli, 2010). Beginning in the third decade, total testosterone concentrations decline at a rate of ~1% per year, simultaneously with a decrease in free testosterone and albumin-bound testosterone at ~2% year (Feldman et al., 2002). During menopause women undergo significant hormonal alterations, of which the decline in oestrogen is thought to have substantial consequences for women's health; specifically, the accelerated loss of bone mineral density and subcutaneous fat accumulation (Messier et al., 2011). The canonical hormonal alterations observed with ageing have consequences for skeletal muscle. Reduced levels of free testosterone are associated with muscle mass loss (Yuki et al., 2013) and higher levels of total testosterone are associated with less muscle mass loss (LeBlanc et al., 2011), which may have significant consequences for the maintenance of physical function in older adults. It is well

documented that supraphysiological exogenous testosterone supplementation can enhance skeletal muscle mass and strength in young (Bhasin et al., 1996) and older men (Ferrando et al., 2002, Neto et al., 2015). Even restoring testosterone in men aged 65 years to the mid-normal range of values for a young individual resulted in significant improvements in muscle mass (Snyder et al., 1999). Thus, hormonal modifications with advancing age may be an important intervention point for preventing declines muscle mass and physical function and promoting the extension of the healthspan.

1.3.5.4. Intramuscular Anabolic Signalling

It has been repeatedly demonstrated that ageing induces a dysregulation of intramuscular anabolic signalling (Guillet et al., 2004, Cuthbertson et al., 2004, Kumar et al., 2009, Fry et al., 2011). Guillet and colleagues were the first group to demonstrate that rates of mixed muscle and mitochondrial protein synthetic rates were lower in older individuals in a fasted state and in response to a hyperinsulinemic, euglycemic, hyperaminoacidemic clamp compared with younger individuals (Guillet et al., 2004). The observed deficit in MPS response occurred in association with a reduction in the degree of activation, by phosphorylation, of p70S6K. However, other signalling proteins (i.e., 4E-BP1, mTORC1 and Akt) responded normally. In an attempt to undertake a more physiologically applicable investigation, insulin was clamped at basal levels and 10g of EAA were provided to establish whether the previously observed differences in MPS persisted between young and old. A blunted myofibrillar and sarcoplasmic MPS response was found in older individuals, concomitant with deficits in mTORC1 and p70S6K phosphorylation (Cuthbertson et al., 2004). Moreover, the acute MPS and intramuscular signalling response to resistance exercise across a spectrum of resistance exercise

intensities, showed that basal rates of MPS did not differ between young and old, however a sigmoidal dose-response relationship was noted with the overall MPS response being 30% greater in the young compared with the old (Kumar et al., 2009). In addition, the FSR peak at 1-2 hours' post-exercise was significantly greater in the young, and this was supported by significantly greater levels p70S6K and 4E-BP1 phosphorylation at 1-hour post-exercise. An acute bout of resistance exercise upregulated mixed muscle FSR in young, but not old, individuals and this occurred concurrently with enhanced phosphorylation of anabolic signalling markers implicated in skeletal muscle translational regulation (ribosomal protein S6 (rpS6), extracellular-regulated signal kinase 1/2 (ERK1/2), mTORC1, p70S6K, 4E-BP1) (Fry et al., 2011). Thus, altered intramuscular signalling occurs concomitantly with age-associated blunting in MPS.

1.3.5.5. Physical (In)activity and Disuse

Reductions in physical activity are commonplace amongst the ageing community. It is recommended that older adults should undertake multicomponent physical activity that includes balance training as well as aerobic and muscle-strengthening activities (chronic conditions permitting) (Piercy et al., 2018). Recent estimates suggest that ~80% of adults in America are insufficiently active (Piercy et al., 2018). Similarly, 15% of older men and 10% of older females achieved the recommended physical activity guidelines when measured objectively by accelerometry (Jefferis et al., 2014). The lack of people meeting the recommended physical activity guidelines has dire consequences for the risk of developing disabling health conditions and premature mortality (Lee et al., 2012). The gradual declining physical activity levels punctuated by protracted periods of disuse in older populations are fundamental drivers of accelerated skeletal muscle atrophy (Alkner

and Tesch, 2004). As little as two weeks of reduced activity (undertaking ~1400 steps per day) was enough to induce anabolic resistance to feeding, of 25 egg white protein, and reduce leg lean mass by ~4% (Breen et al., 2013). Similar observations occur when physical activity is reduced by disuse (Wall et al., 2013) and immobilisation (Glover et al., 2008). A reduction of muscle mass with immobilisation has been demonstrated in both young (Glover et al., 2008), and older (Breen et al., 2013) populations, and seems to be driven by reductions in postprandial MPS. The loss of muscle mass does not seem to be determined by alterations to basal postabsorptive MPS (Breen et al., 2013), and current evidence indicates older individuals demonstrate a blunted response to anabolic stimuli (i.e., exercise or feeding). Periods of immobilisation (i.e., bed rest, limb casting) and reductions in physical activity (i.e., sedentariness, reduced step-count), characteristics commonly observed in ageing populations, have devastating consequences for skeletal muscle (Dirks et al., 2016). One week of bed rest is enough to induce whole-body insulin resistance, reductions in muscle oxidative capacity and significantly decrease skeletal muscle mass and strength by 4 and 9 %, respectively (Dirks et al., 2016). During these periods MPB exceeds MPS, due to an increase in MPB or a decrease in MPS, or both and the problem is exacerbated by the inability to recover the responsiveness to anabolic stimuli following relatively short periods of disuse. When the daily activity of a group of older men and women was reduced to ~1000 steps per day (McGlory et al., 2018), the change in activity status was sufficient to induce a significant reduction in MPS. Which upon re-ambulation did not recover, following two weeks of returning to normal activity, demonstrating how damaging periods of disuse are for muscle mass maintenance.

Physical activity may play an important role in preventing anabolic resistance, augmenting the MPS response and potentially slowing the progression of sarcopenia.

Acutely, when performing combined aerobic and resistance exercise prior to the ingestion of 20g protein the MPS response increased significantly compared with resting rates; importantly, this occurred irrespective of age (Pennings et al., 2011). However, it is yet to be determined whether older individuals that have continuously performed extremely high levels of physical activity for prolonged periods (i.e., years) are able to offset declines in anabolic sensitivity and MPS. The study of individuals that have done so would enable researchers to determine whether the observed deterioration is purely an effect of ageing or the consequence of physical inactivity.

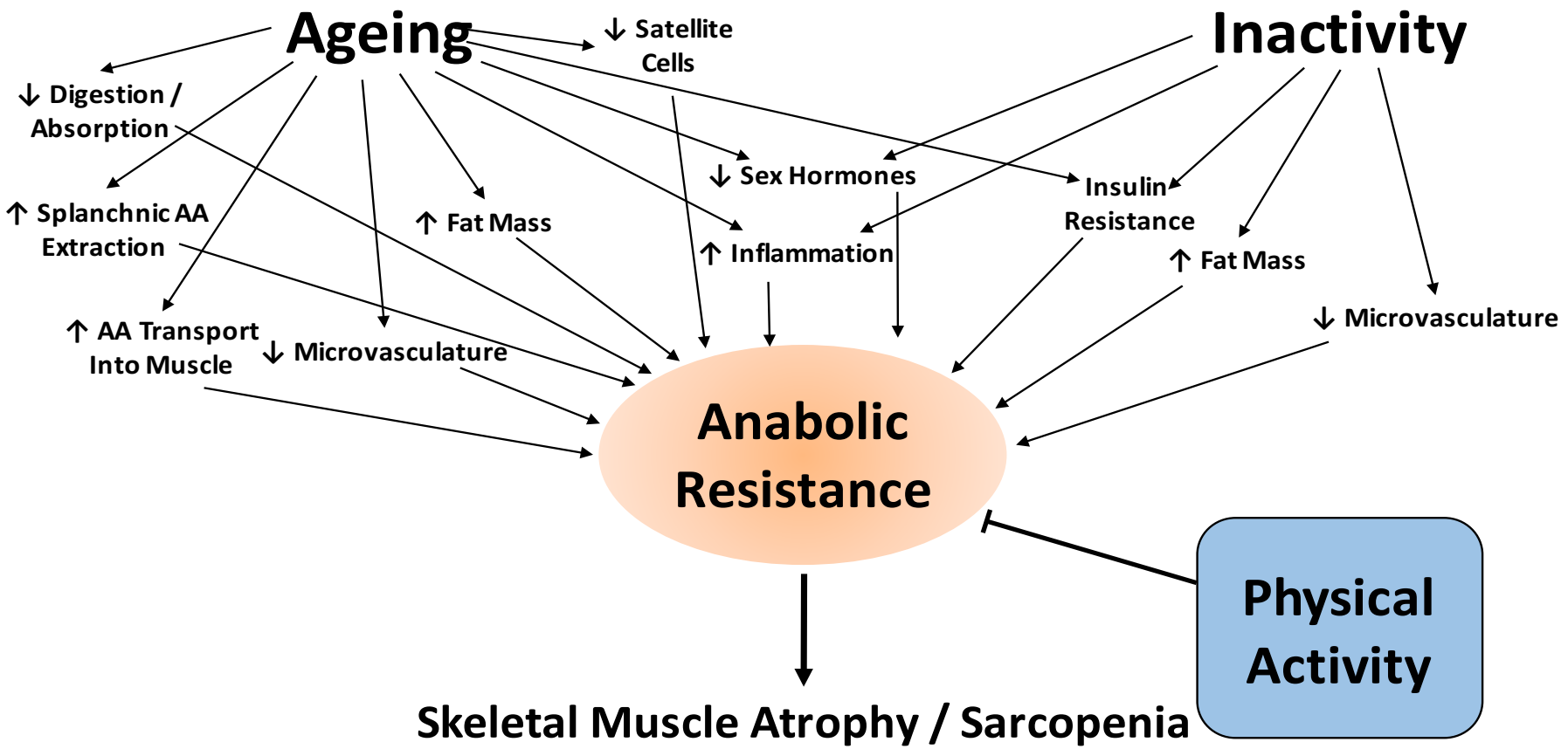

Figure 3. Anabolic resistance is considered an important driver of skeletal muscle mass loss with ageing (sarcopenia). This figure illustrates a simplification of the complex network of underlying factors that contribute to the development of anabolic resistance.

1.4. The Master Athlete: A Model for Healthy Musculoskeletal Ageing?

The study of mechanisms of skeletal muscle ageing in a human population is problematic. Advances in technology and improvements in infrastructure have led to reductions in physical activity gradually since the 1900s (Booth and Lees, 2006). Presently, the strain placed on global healthcare systems necessitates interventions that promote extension of the healthspan. Primary ageing is often confounded by environmental influences which can accelerate the erosion of physiological function and health (Lazarus and Harridge, 2007). Frank Booth elegantly stated that *"Being physically active is the body's biological default position to maintain physiological function throughout the life course" (Harridge and Lazarus, 2017)*. Thus, in order to unravel the impact that primary ageing and alterations to physical activity status elicit on skeletal muscle function, mass, and morphology, it is imperative that an appropriate model is implemented. However, an important consideration in the study of MA is the selection bias that may limit the translatability of research findings to the wider populations. Specifically, not only are MA highly active, MA often demonstrate superior nutritional habits, a higher socioeconomic background, better education and may be genetically predisposed to superior physical function (Hannam et al., 2017). The recruitment of MA in older age also selectively employs those that have avoided serious injury or illness. Nevertheless, MA offer an exciting opportunity to investigate proposed 'optimal ageing', distinct from canonical increases in physical inactivity.

1.4.1. Definition and Performance Characteristics of Master Athletes

Individuals, typically ~35-80 y, that have continued to undertake structured exercise training and competition, are commonly referred to as 'Master Athletes' (MA)

(sometimes ‘veteran or senior athletes’). As physical activity is known to have a profound effect of physiological function, skeletal muscle mass and morphology, intuitively, older individuals that have maintained high levels of exercise throughout life are likely to display a superior range of physiological function and health parameters compared with older non-exercising individuals (Lazarus and Harridge, 2010). This poses the question of whether the continuation of exercise training throughout the life-course can offset age-related impairments in skeletal muscle function, mass and morphology and subsequently extend the healthspan. The proposed decline in physiological function with increasing age and levels of physical activity (i.e., in sedentary and habitually active older individuals and MA) is depicted in Figure 4.

The inclusion of MA in physiology research is not a novel idea. Some of the first work to incorporate physically well trained older athletes dates back to 1966, when it was shown that VO_{2max}, along with other measures of cardiovascular physiology, declined with advancing age, despite continuous endurance training (Grimby and Saltin, 1966). A key focus of MA research studies since has been to examine world record performances across the age spectrum in these well-trained older individuals, to provide insight into the maximal capacity for successful integration of all bodily systems (Harridge and Lazarus, 2017). As a result, there are substantial data illustrating that world record performances decline linearly with age until approximately the 8th decade, following which the rate of decline is greatly accelerated (Baker et al., 2003, Baker and Tang, 2010, Rittweger et al., 2009, Tanaka and Seals, 2003). Nevertheless, world record performances in older athletes are astounding. For example, the current world record for i) 100 m sprint time for a 75-year-old is 13.49 and 15.03 s for men and women, respectively, ii) 10,000 m for an 80-year-old is 42:39 and 51:46 for men and women, respectively. It appears that chronic

training in MA does not necessarily help these individuals escape the decline in physiological function and health associated with inherent ageing. Nevertheless, advancements in experimental methodology and analytical techniques has enabled researchers to better understand the extent to which chronic exercise training offsets decrements in physiological function and performance in old age, and shed light on the mechanisms responsible for these alterations.

1.4.2. Muscle Morphology and Function in Master Athletes

Individuals that have continued to exercise throughout their lifespan consistently demonstrate superior physiological function (i.e., VO_{2max}, muscular strength, physical function) compared with non-exercisers. Senior sportsmen exhibit greater muscular strength and physical performance than their older sedentary (untrained) counterparts, comparable to that of young healthy individuals (Zampieri et al., 2015). Nevertheless, MA still demonstrate a decline in VO_{2max} (Faulkner et al., 2008, Wilson and Tanaka, 2000, Fitzgerald et al., 1997, Tanaka et al., 1997) and muscular strength/power (Goodpaster et al., 2006, Rittweger et al., 2009, Faulkner et al., 2008). Crucially, MA have a much larger physiological reserve capacity, thereby allowing for greater alterations to physical function before independence and quality of life are compromised. Given the importance of physical function and skeletal muscle health during the ageing process, it can be assumed that MA are far more likely to advance through later years without the presence of disease and disability. To date, our understanding of the mechanisms that dictate the age-associated alterations in physical function in MA are still somewhat limited.

In addition to studying the deterioration of physiological function in MA, the effect of exercise training throughout the life-course may offset the decline in skeletal muscle mass. However, there is no clear consensus regarding the impact that chronic exercise training

exerts on skeletal muscle mass maintenance. Previously, differences in skeletal muscle mass between endurance-trained MA and age-matched sedentary individuals have been undetectable (Carrick-Ranson et al., 2014). It has been shown that chronic endurance exercise training is able to preserve skeletal muscle mass (Wroblewski et al., 2011) and master rowers have greater total and regional lean mass compared with age-matched sedentary counterparts (Sanada et al., 2009). Conversely, older endurance trained men and women have significantly less fat-free mass than their younger endurance trained comparators (Proctor and Joyner, 1997). In summary, the effect of chronic endurance training on muscle mass maintenance appears equivocal. Conversely, it has been demonstrated that strength trained MA have significantly more lean body mass and greater strength than untrained individuals of the same age (Sallinen et al., 2008). However, the cross-sectional nature of this study prevents firm conclusions being drawn as to whether strength training prevent muscle mass loss in MA. Which may suggest, the maintenance of skeletal muscle mass with advancing age is dependent on the exercise training modality (resistance vs. endurance). Therefore, research stratifying the effects of chronic exercise training mode on muscle mass maintenance is of foremost importance.

Similar to skeletal muscle mass, there is much debate about the effect of chronic exercise training on skeletal muscle architecture (Korhonen et al., 2006, Ojanen et al., 2007) and fibre morphology (Korhonen et al., 2006, Aagaard et al., 2007a) in MA. Skeletal muscle architecture has previously been shown to be altered with ageing (Narici et al., 2003, Strasser et al., 2013). Despite continuous sprint training, *Vastus Lateralis* thickness is reduced with ageing, with no effect on fascicle length (Korhonen et al., 2006). Further, it has been shown that, relatively young, master throwers had significantly greater muscle thickness than older master throwers (Ojanen et al., 2007). These data indicate that

although muscle thickness decreases with advancing age, continuous resistance training may cause an upward shift in the trajectory of decline. That said, it appears endurance running in older age has little effect on skeletal muscle architecture (Stenroth et al., 2016). Alterations to muscle architecture have significant consequences for skeletal muscle function. As a result, more conclusive investigations into the effect that chronic exercise has on skeletal muscle architecture in MA are needed.

The muscle loss experienced during ageing can largely be attributed to a Type II fibre atrophy (Nilwik et al., 2013) and fibre loss (McPhee et al., 2018). Due to the generally well accepted relationship between muscle cross-sectional area and the force generating capacity of muscle (Maughan, 1984), this may have severe functional implications. Whether or not the continuation of exercise in MA is able to prevent or attenuate muscle fibre atrophy, is much less clear. Strength trained older individuals had larger Type I fibres than endurance trained older individuals, and larger Type IIa and IIx fibres than untrained and endurance trained older individuals (Aagaard et al., 2007a). In addition, the distribution of the fibre-type reflected the training modality, with endurance trained older individuals displaying a greater proportion Type 1 fibres than untrained and strength trained older individuals. Whereas, others have been unable to detect any difference in fibre CSA between young, old, untrained old and master endurance runners (Mackey et al., 2014). However a recent study showed that young individuals had larger fibre diameter than senior sportsmen, from varied training backgrounds, and the senior sportsmen had a greater fibre diameter compared with sedentary seniors (young > senior sportsmen > sedentary seniors) (Zampieri et al., 2015). Plausibly, this may suggest that the continuation of exercise into later years can offset some of the age-related declines in fibre CSA, but not prevent them; though this requires further confirmation.

Beyond skeletal muscle mass, architecture and fibre CSA in MA, there is a paucity of studies investigating skeletal muscle microscopic and neuromuscular properties in this population. Two studies investigating the effects of ageing and chronic exercise on neuromuscular structure and function report conflicting findings. Initially it was shown that master runners were able to maintain the estimated number of motor units (MUNE), despite a reduced strength output, when compared with untrained young and older individuals (Power et al., 2010). However more recently, others have been unable to replicate this and instead report that chronic exercise training in master runners was not preventative of the decline in MUNE (Piasecki et al., 2016a). The discrepant findings between these studies are difficult to reconcile as both studies investigated the same muscle, using similar analytical methods. It may be that chronic exercise training, in MA, is able to protect other muscle fibre properties (i.e., capillarisation, satellite cells) from deterioration with older age. For example, Coggan et al. (1990) showed that muscle fibre capillarisation in MA was similar when compared with competitive young runners, however this did not prevent a reduction in VO_{2max} in MA (Coggan et al., 1990). More recently these findings have been reinforced by work demonstrating that 50+ years of continuous endurance training preserved skeletal muscle capillarisation (Gries et al., 2018). Again, it was shown that the decline in physiological function, in this case VO_{2max}, was offset in the highly trained older individuals (i.e., young > MA > non-exercising older individuals). A gradual decline in capillarisation has been demonstrated in MA between ~55 and 80 years, which is likely reflective of an inherent age-related decline (Pollock et al., 2018). Furthermore, only one study has investigated satellite cells in MA, and they highlight no differences in satellite cell content regardless of age or training status (Mackey et al., 2014). Which is perhaps surprising as studies often report a reduction of

Type II fibre specific satellite cell content. As a result, it appears that further research is required to fully elucidate the effect that the continuation of chronic exercise training exerts on the microscopic properties of skeletal muscle fibres (i.e., fibre CSA, capillarisation). Likewise, a number of morphological features require further investigation (i.e., myonuclei, satellite cells) in this unique population. This will deepen our understanding on the effects of inherent ageing on muscle morphological features, and shed light on the extent to which exercise can protect against losses that underpin physiological function.

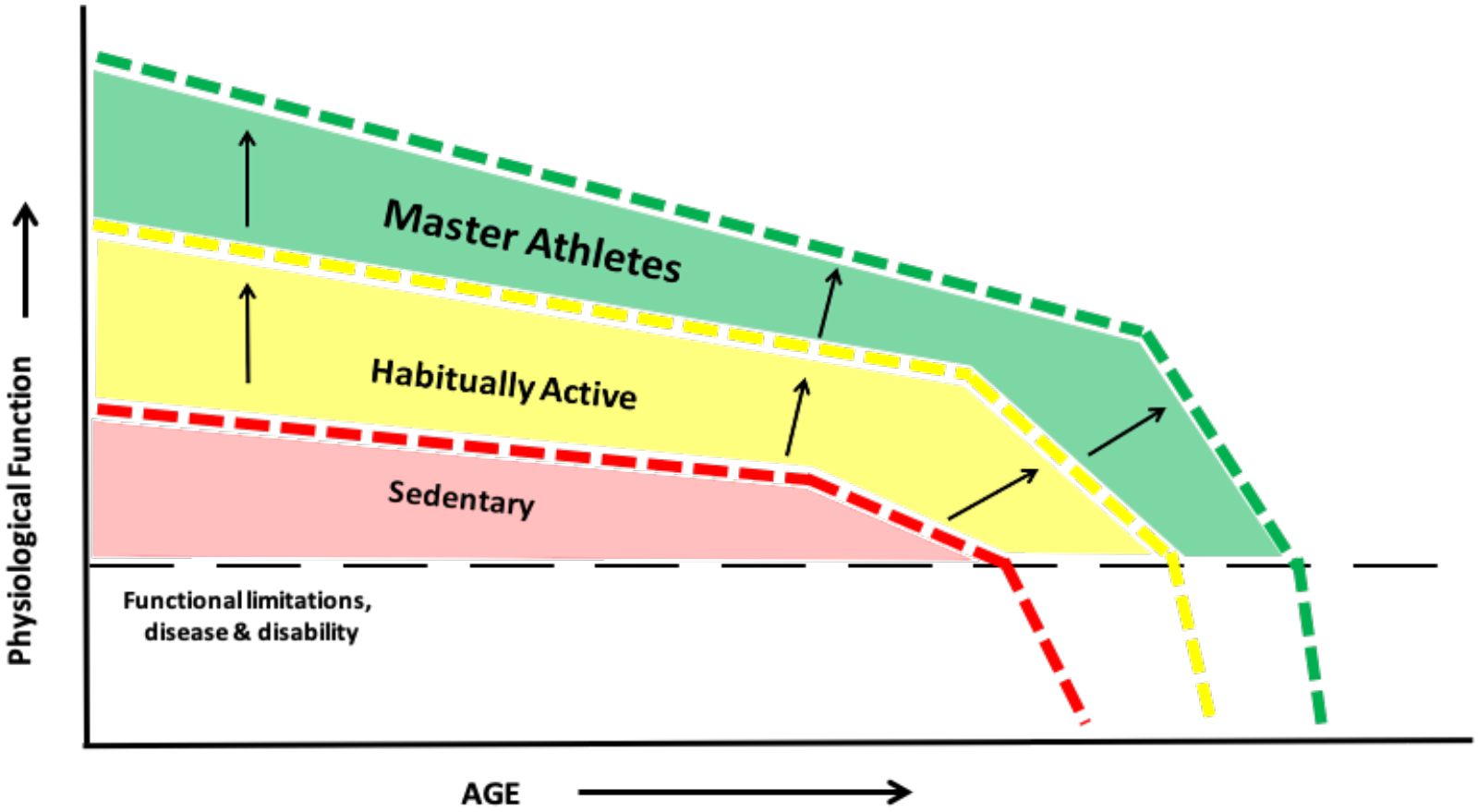

Figure 4. Physiological function deteriorates with ageing, the portion of life that remains above the line for 'Functional limitations, disease and disability' represents healthspan. Increasing activity levels lead to an upward and rightward shift in the decline of physiological function, which reflects an increased period of time individuals experience life unburdened by poor health and functional limitations (healthspan). Figure adapted and redrawn from Seals et al. (2015).

1.4.3 Muscle Protein Turnover in Master Athletes

The maintenance of regulatory process that govern skeletal muscle turnover is critical with advancing age. Previously, only one study has investigated the impact that the maintenance of exercise training into later years exhibits on skeletal muscle protein metabolism (Doering et al., 2016). This study showed that master triathletes had lower (1.49%·d^{-1}) iMPS rates than young triathletes (1.7%·d^{-1}), measured over 3 d of consecutive training, in response to an initial downhill running exercise stimulus. In light of this, despite the continuation of exercise training in older age, MA may exhibit an impairment in the muscle remodelling process though this is yet to be confirmed. However, this was also reflected in the greater attenuation of performance in MA in response to the initial exercise bout. Nevertheless, the incorporation of an un-trained older cohort for comparison against MA, would have enabled the researchers to distinguish whether the observed differences in MPS, were the result of inherent ageing or due to aspects of secondary/biological ageing. Further, it is essential that future studies include a basal measurement period of iMPS and confirm whether the muscle responsiveness to a bout of resistance exercise to describe the net muscle anabolic effect of exercise (i.e. difference between rest and exercise-induced iMPS).

1.5. Thesis Aims and Objectives

It is apparent that ageing exerts deleterious effects on skeletal muscle metabolism, morphology and function, that appear to be underpinned, in part, by inherent ageing processes and aspects of secondary/biological ageing. MA are a model of inherent ageing free from the confounding factor of biological ageing (i.e. inactivity), and generally display superior physiological function compared with their untrained age-matched

counterparts. The mechanisms underpinning the benefits of chronic exercise training with advancing age have been only partially explored, indicating that the 'normal' age-related deterioration of physical function, muscle mass and morphology appears to be offset (shifted up and to the right). However, deeper insight into the regulatory processes that may protect MA muscle are less clear. The overarching aim of this thesis is to identify and address knowledge gaps regarding the impact that chronic exercise training exerts on skeletal muscle metabolism, morphology and function and to improve understanding of the mechanisms that govern these processes in MA. The specific aim of each thesis chapter is described below.

First, the absence of a narrative/systematic review highlighted the need for a thorough synthesis and evaluation of the current status of the literature surrounding MA, and the extent to which chronic exercise training may preserve indices of skeletal muscle mass, function and morphology. Therefore, the focus of **Chapter 2** was to identify what is already known regarding whether MA preserve physical function, muscular strength, mass and fibre properties compared with untrained age-matched individuals, as well as younger trained and untrained individuals.

Second, although previous cross-sectional studies have been conducted in MA, to date, there are very few high-quality comprehensive cross-sectional studies characterising the physiological function, muscle mass and morphology in chronically trained MA. Thus, **Chapter 3** reports a cross-sectional study that investigated whether MA have superior functional capacity, cardiorespiratory fitness, body composition, muscle strength, architecture and fibre-type properties compared with untrained healthy younger and untrained age-matched individuals.

Third, only one previous study has investigated whether the continuation of exercise training into older age is able to preserve muscle anabolic sensitivity to an exercise stimulus. However, a primary focus of the previous investigation was recovery from damaging exercise (i.e., downhill running) and it was not established whether differences exist in basal rates of iMPS, or whether differences exist between MA and older untrained individuals in response to an anabolic (resistance exercise) stimulus. Therefore, this area of research requires significant attention, and **Chapter 4** reports a study that investigated whether basal and resistance exercise-induced iMPS differs between MA and age-matched untrained individuals, and characterised the acute intramuscular mechanisms coordinating the response.

Finally, **Chapter 5** discusses the main findings of the preceding chapters and details appropriate conclusions about the impact chronic exercise training, in MA, has on indices of skeletal muscle metabolism, morphology and function. Finally, this chapter identifies the practical implications and makes recommendations for future research directions.

2. Muscle Morphology and Performance in Master Athletes: A Systematic Review and Meta-analyses

2.1. ABSTRACT

Introduction: The extent to which chronic exercise training preserves age-related decrements in physical function, muscle strength, mass and morphology is unclear. Our aim was to conduct a systematic review of the literature to determine to what extent chronically trained master athletes (strength/power and endurance) preserve levels of physical function, muscle strength, muscle mass and morphology in older age, compared with older and younger controls and young trained individuals.

Methods: The systematic data search included Medline, EMBASE, SPORTDiscus, CINAHL and Web of Science databases. ***Inclusion criteria***: i) master athletes mean exercise training duration ≥20 years ii) master athletes mean age of cohort >59 years) iii) at least one measurement of muscle mass/volume/fibre-type morphology and/or strength/physical function.

Results: Fifty-five eligible studies were identified. Meta-analyses were carried out on maximal aerobic capacity, maximal voluntary contraction and body composition. Master endurance athletes (42.0 ± 6.6 ml·kg^{-1}·min-1) exhibited VO_{2max} values comparable with young healthy controls (43.1 ± 6.8 ml·kg^{-1}·min^{-1}, P=0.84), greater than older controls (27.1 ± 4.3 ml·kg^{-1}·min^{-1}, P<0.01) and master strength/power athletes (26.5 ± 2.3 ml·kg^{-1}·min^{-1}, P<0.01), and lower than young endurance trained individuals (60.0 ± 5.4 ml·kg^{-1}·min^{-1}, P<0.01). Master strength/power athletes (0.60 (0.28 to 0.93) P<0.01) and young controls (0.71 (0.06 to 1.36) P<0.05) were significantly stronger compared with the other groups. Body fat % was greater in master endurance athletes than young endurance trained (-4.44% (-8.44 to -0.43) P<0.05) but lower compared with older controls (7.11% (5.70 to 8.52) P<0.01).

Conclusion: Despite advancing age, this review suggests that chronic exercise training preserves physical function, muscular strength and body fat levels similar to that of young, healthy individuals in an exercise mode-specific manner.

2.2. INTRODUCTION

The UK population is projected to increase by ~25% between 2013 and 2060, from 64.1 to 80.1 million (Mitchell et al., 2012b) which heralds a demographic shift towards an ageing society. This changing demographic presents a significant and overwhelming challenge to healthcare provision in the UK (Holloszy, 2000). Indeed, although individuals are living longer (i.e., lifespan), many endure a large portion of their later years with a number of age-related comorbidities (Seals et al., 2016). Extending the length of time individuals remain healthy and disease-free (i.e. health-span) with an emphasis on compressing morbidity is therefore an important focus (Seals et al., 2016). Physical function (e.g., aerobic capacity and muscular strength) typically declines with advancing age and this is often highlighted as a principal risk factor for the development of a number of degenerative chronic health conditions (Niccoli and Partridge, 2012). However, it has been suggested that exercise throughout the lifespan (i.e., 'chronic' exercise training) can attenuate or even prevent age-related declines in physical function. Understanding whether, and to what extent, chronic exercise training preserves physical function, muscle strength, mass and morphology is of great importance in the pursuit of appropriate countermeasures to age-related health deterioration.

Reductions of aerobic capacity (VO_{2max}) and muscular strength are major risk factors for all-cause mortality in older age (Ortega et al., 2012, Ruiz et al., 2008, Lee et al., 2011). VO_{2max} and muscular strength are often considered robust measures of physical function and health as they require successful integration of the cardiovascular, respiratory and neuromuscular systems (Harridge and Lazarus, 2017). The gradual decline of these bodily systems with ageing ultimately reduces the ability of older individuals to carry out activities of daily living (ADL); forcing many into a state of reduced physical

independence and a poorer quality of life (Sonn, 1996). Delineating the relative contribution of primary ageing and environmental influences (or secondary ageing) to the age-related decline in physical function, muscle strength, mass and morphology is problematic. One aspect of secondary aging that is considered to be particularly influential is habitual physical activity. The majority of older adults reduce habitual physical activity with advancing age (Blair, 2009), and this is often accompanied by the presence of at least one chronic disease (Hung et al., 2011). However, a small sub-set of the population, referred to as master athletes, are unique in that they have chronically undertaken and continue to maintain high levels of physical activity, including structured exercise training. Indeed, Zampieri and colleagues (Zampieri et al., 2015) demonstrated that senior sportsmen from varied training backgrounds exhibited muscular strength, performance, myofibre properties and function comparable with young, healthy individuals. These findings suggest that chronic exercise training can preserve physical function and skeletal muscle properties in older age. As such, the study of Master athletes may allow us the opportunity to distinguish the contribution of primary and secondary ageing to the age-related decline in health, function and performance (Harridge and Lazarus, 2017, Lazarus and Harridge, 2007). However, Mackey and colleagues observed no difference in type I or type II fibre size between young and old regardless of training status, despite differences in VO_{2max}; making it challenging to draw firm conclusions regarding the impact ageing and/or chronic endurance exercise elicits on fibre area (Mackey et al., 2014). To further complicate the variable findings in these unique individuals, Piasecki and colleagues demonstrated that the loss of muscle size, strength and motor units in the *Tibialis anterior* was similar between master endurance athletes and age-matched untrained controls (Piasecki et al., 2016a). The inconsistent findings highlighted advocate the need for a

quantitative summary of the existing literature surrounding the effect of chronic exercise training on indices of performance and skeletal muscle properties.

Earlier comparisons between young and older individuals and master athletes have typically included master athletes younger than 60 y (Gent and Norton, 2013, Kusy and Zielinski, 2014, Maffulli et al., 1994). Specifically, this systematic review will focus on master athletes 60 y or older as these individuals would typically have begun to experience age-related decrements in physical function, muscle strength, mass and morphology (Janssen et al., 2000, Doherty, 2003). Additionally, most systematic review comparisons between young and older individuals and master athletes have focused on single outcome measures, specifically body composition (Ballor and Keesey, 1991), aerobic capacity (Fitzgerald et al., 1997, Wilson and Tanaka, 2000), muscular strength (Peterson et al., 2010) adaptations to training (Daskalopoulou et al., 2017) and protein supplementation (Doering et al., 2015). Therefore, the primary aim of this systematic review was to establish whether older individuals who have undertaken chronic exercise training, preserve physical function, muscular strength, mass and fibre properties (i.e. size and relative distribution) compared with untrained age-matched individuals, as well as younger trained and untrained individuals. A secondary aim was to determine the influence of exercise modality (i.e., strength/power vs. endurance) on the included parameters.

2.3. METHODS

2.3.1. Information sources and literature search

A systematic literature search of online databases was conducted in November 2017 using selected key words, free text terms, indexed terms, and Boolean operators. The search strategies were applied to Medline, EMBASE, SPORTDiscus, CINAHL and Web of Science databases. Recursive searching of the bibliographies of eligible studies and relevant reviews was performed to identify additional articles. The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) report (Moher et al., 2009).

2.3.2. Study Selection Criteria

2.3.2.1 Inclusion Criteria

Studies were included in the review if they met the following inclusion criteria: 1) Exercise training for a minimum of 20 years in the master athletes group, explicitly stated within the study. 2) Mean age of older cohorts older than59 years. 3) Inclusion of at least one measurement of muscle mass/volume/fibre type morphology (fibre type, size, area) and/or a measurement of strength/physical function. 4) Muscle mass measurement using magnetic resonance imaging (MRI), computed tomography (CT), dual x-ray absorptiometry (DXA), air displacement plethysmography (BodPod), hydrostatic weighing, bioelectrical impedance analysis (BIA) or B-Mode ultrasonography. Physical function/strength/performance measurements to include one, or more, of: handgrip strength, isometric/isotonic strength/power/torque or aerobic capacity (VO_{2max}). 5) Freedom from any neurological, neuromuscular, cardiovascular and metabolic disease. 6) Studies published only in English with no date restrictions.

2.3.2.2. Exclusion Criteria

Studies were excluded from the review if: 1) The authors did not explicitly state the length of time that master athletes had been continuously training, or if that time was less than 20 years. 2) No inclusion of relevant measures of muscle mass/morphology or physical function. 3) Relevant data could not be obtained directly from potentially eligible articles or via contact with the study authors.

2.3.3. Participant criteria

Sedentary older control groups were required to be of a similar age to master athlete groups (mean group >59 years) and have undertaken little, to no, structured exercise training. Older control groups did not have to be completely sedentary compared with master athlete groups. Young controls were required to be younger than 40 years, and have undertaken little to no structured exercise training. Young controls did not have to be completely sedentary compared with young trained individuals, who were required to be younger than 40 years and undertaking either structured endurance or strength/power-based exercise training.

2.3.4. Study Selection

Titles and abstracts were screened for relevance by 2 reviewers (J.M. and B.J.S). Irrelevant titles were removed. Full-text articles were obtained for potentially relevant studies via a combination of online databases, hardcopy sourcing and direct contact with the authors, and these were further evaluated to determine whether they met the inclusion criteria. Studies deemed eligible were included in the systematic review. Two reviewers

(J.M and B.J.S.) independently assessed full-texts for eligibility; any disagreements between the two reviewers were settled by consensus. All records were managed using the reference software EndNote (Thomson Reuters, v.X7).

2.3.5. Data Extraction

Predetermined variables were extracted from each of the included studies using a customised data extraction form (J.M and B.J.S). Measures of interest included participant characteristics (number, age, and anthropometrics), training type and duration, measurements of muscle mass, physical function (strength, aerobic capacity) and fibre-type characteristics along with various aspects of study design/assessment (measurement tool or analytical method). In situations where the necessary data could not be obtained, either directly from the article or by contacting the corresponding authors of the paper, the study (or particular outcome measure) was excluded from review and/or meta-analysis. Where BMI was not reported, it was calculated manually from the reported mean height and weight of the study groups.

2.3.6. Data Syntheses & Statistical Analyses

Statistical analyses were dependent upon the number of studies obtained and the associated outcomes measures in the relevant studies. If sufficient studies incorporating an eligible analytical protocol were identified for a single outcome measure (e.g., VO_{2max}), a meta-analysis was conducted. In cases where insufficient data were available from eligible studies to conduct a meta-analysis for a single outcome measure (e.g., muscle mass), findings were presented narratively/qualitatively.

Meta-analyses were performed on studies (and variables) which included direct comparisons (within study) between master athletes (power/endurance) and a non-exercising older controls (older control) or young trained/non-trained groups. Meta-analyses were carried out with a random effects model using RevMan software (Review Manager (RevMan) V.5.3. Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Data in meta-analyses were presented as mean differences (MD)/standardised mean differences (SMD) ± 95% confidence interval (CI). Standardised mean differences were presented only for outcomes reported using non-comparable scales (i.e. muscular strength). All data were presented as mean ± SD, along with the number of studies/study groups reporting that particular variable and the number of participants included in that comparison.

2.4. RESULTS

2.4.1. Study Selection

A total of 14,572 studies were identified by the extensive literature search and a further 1 study was identified by manual searching of reference lists. Following removal of duplicates, 393 study titles and abstracts were screened resulting in 143 articles being excluded from the review; leaving 250 full texts to be independently screened (J.M and B.J.S). Figure 5 highlights the study identification, screening process and reasons for study exclusion.

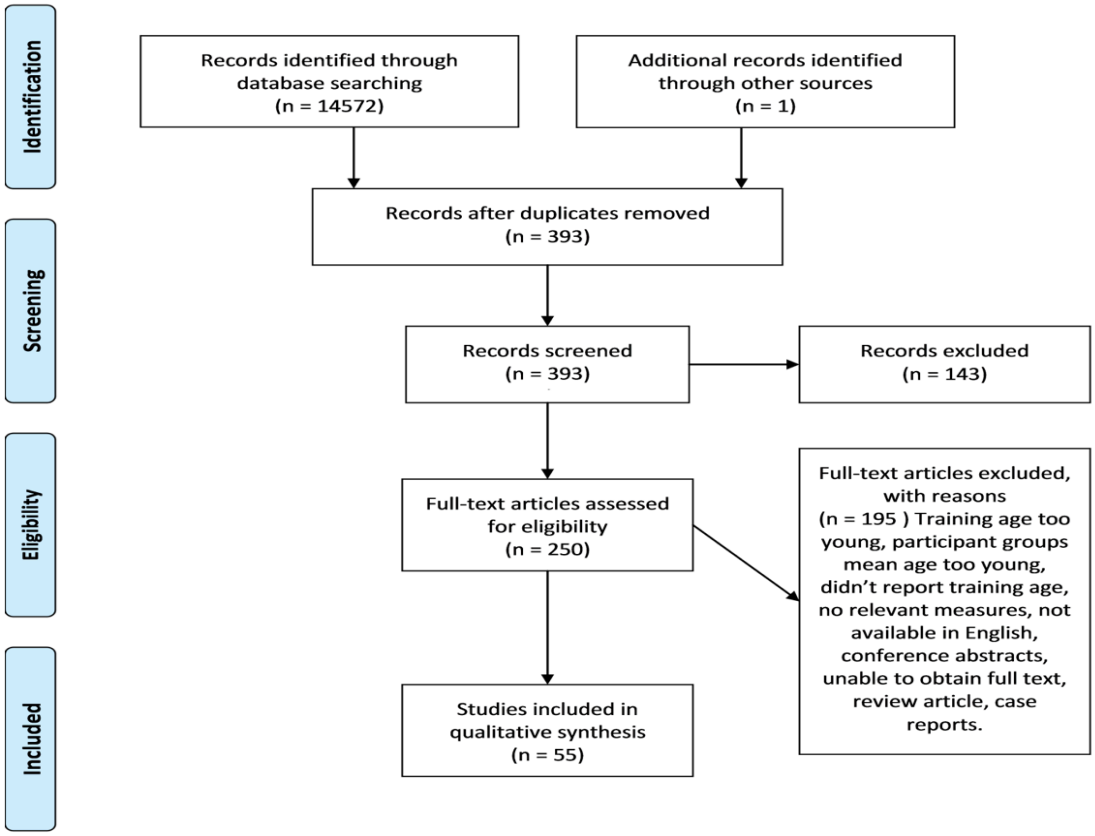

Figure 5. PRISMA flowchart detailing the article identification, screening and exclusion process.

2.4.2. Study Characteristics

The 55 included studies demonstrated substantial heterogeneity regarding the outcome measures and methodology (Table 1). Further, the participant characteristics displayed substantial incongruity, specifically the mode and level of exercise training, and the number of years training experience (Table 2). Twenty-one studies included young untrained control groups, 43 studies included 44 older control groups, 2 studies each included a young strength/power trained group, 11 studies included 12 endurance trained young groups and all studies included at least one master endurance and/or strength/power training group.

2.4.3. Participant Characteristics

The 55 eligible studies included 2449 participants (2181 were Male and 268 Female). In short, included groups were master endurance athletes (67.3 ± 5.1 years, 1.73 ± 0.04 m, 71.6 ± 5.2 kg, 56 study groups, n=958) and master power athletes (71.7 ± 4.8 years, 1.73 ± 0.03 m, 76.2 ± 8.0 kg, 15 study groups, n=218), older controls (67.9 ± 4.5 years, 1.73 ± 0.03 m, 78.2 ± 6.2 kg, 44 study groups, n=746), young endurance trained (25.4 ± 3.1 years, 1.79 ± 0.03 m, 70.1 ± 5.0 kg, 13 study groups, n=143), young power trained (26.0 ± 1.6 years, 1.80 ± 0.02 m, 77.0 ± 0.4 kg, 3 study groups, n=47) and young controls (26.6 ± 3.9 years, 1.79 ± 0.03 m, 75.9 ± 5.0 kg, 23 study groups, n=337). The participant characteristics and anthropometrics of the sub-groups included in the review are outlined in detail in Table 2.

2.4.4. Training characteristics

Experience – Master athletes had been training for 31.1 ± 9.3 (56 study groups, n=958) and 35.6 ± 9.6 (15 study groups, n=218) years for endurance and strength/power groups, respectively. Young athletes had been training for 6.1 ± 2.6 (13 study groups, n =143) and 11.2 ± 5.5 (3 study groups, n=47) years for endurance and strength/power groups, respectively.

Frequency - The included groups reported similar training frequency 4.6 ± 1.4, 3.6 ± 1.1, 5.0 ± 0.8 and 4.1 ± 2.5 times per week, for master endurance (21 study groups, n=339), master strength/power (7 study groups, n=111), young endurance (4 study groups, n=66) and young strength/power trained groups (2 study groups, n=35), respectively.

Duration – The included groups reported a similar number of training hours per week; master endurance 6.8 ± 3.0 h (19 study groups, n=298), master strength/power 6.3 ± 0.7 h (8 study groups, n=164) young endurance 7.7 ± 3.6 h (6 study groups, n=45) and young strength/power 10.0 ± 2.1 h (2 study groups, n=42), respectively.

Distance - Seventeen study groups of master endurance (44.5 ±10.8 km, n=302) and 3 study groups of young endurance trained athletes (44.7 ± 2.9 km, n=30) completed similar weekly cycling distance.

2.4.5. Fat-Free Mass

A total of 12 studies (Carrick-Ranson et al., 2014, DubÉ et al., 2016, Hawkins et al., 2001, Hayes et al., 2015, Marcell et al., 2003, Marcell et al., 2014, Pollock et al., 2015, Proctor and Joyner, 1997, Sanada et al., 2009, Tarpenning et al., 2004, Trappe et al., 2013,

Yataco et al., 1997) reported indices of whole body fat-free mass (FFM) using DXA, hydrostatic weighing or bioelectrical impedance. Nineteen study groups included master endurance athletes (55.2 ± 6.1 kg FFM, n=366), 5 study groups included older controls (54.5 ± 7.7 kg FFM, n=122), 1 study group included young controls (53.8 ± 5.7 kg FFM, n=23) and 4 study groups included young endurance trained individuals (54.8 ± 7.3 kg FFM, n=56).

2.4.6. Fat Mass

Only three studies (DubÉ et al., 2016, Schmidt et al., 2015, Yataco et al., 1997) reported measures of whole-body fat mass (FM) measured using DXA or hydrostatic weighing. Three study groups included master endurance athletes (14.1 ± 3.6 kg FM, n=91), 2 study groups included older untrained individuals (20.1 ± 6.3 kg FM, n=65) and 1 study group included young endurance trained individuals (12.0 ± 4.2 kg FM, n=14).

2.4.7. Body Fat Percentage

A meta-analysis was conducted for body fat % for studies that made direct comparisons with master endurance athletes (Figure 6); this allowed for the greatest number of within-study comparisons to be incorporated into the statistical analysis. Twenty-nine studies (Aagaard et al., 2007a, Arbab-Zadeh et al., 2004, Ari et al., 2004, Bjork et al., 2012, Buford et al., 2010, Buyukyazi, 2004, Carrick-Ranson et al., 2014, Cristea et al., 2008, DubÉ et al., 2016, Hawkins et al., 2001, Hayes et al., 2015, Katzel et al., 1998, Korhonen et al., 2006, Korhonen et al., 2012, Marcell et al., 2003, Marcell et al., 2014, Matelot et al., 2016, Mortensen et al., 2012, Nyberg et al., 2012, Ojanen et al., 2007,

Pollock et al., 2015, Proctor and Joyner, 1997, Sallinen et al., 2008, Sanada et al., 2009, Schmidt et al., 2015, Tarpenning et al., 2004, Trappe et al., 2013, Witkowski et al., 2010, Yataco et al., 1997) reported data for body fat percentage measured using bioelectrical impedance, DXA, hydrostatic weighing or multiple-site skinfold. Thirty study groups included master endurance athletes (19.7 ± 3.8 %, n=594), 11 study groups included master strength/power athletes (16.4 ± 4.4 %, n=136), 19 study groups older control (24.5 ± 4.6 %, n=361), 5 study groups included young endurance trained (15.4 ± 5.2 %, n=63), 2 study groups included young strength/power trained individuals (14.1 ± 3.5 %, n=42) and 8 study groups included young controls (17.4 ± 3.0 %, n=126).

Master endurance athletes had a significantly lower body fat % than older control individuals ($P<0.01$), and a significantly greater body fat % than young endurance trained ($P<0.05$). No significant differences were observed in the body fat % between master endurance athletes when compared with young controls and master strength/power athletes. Young strength/power trained individuals were not included in the meta-analyses as no eligible studies made direct within-study comparisons with the other included groups.

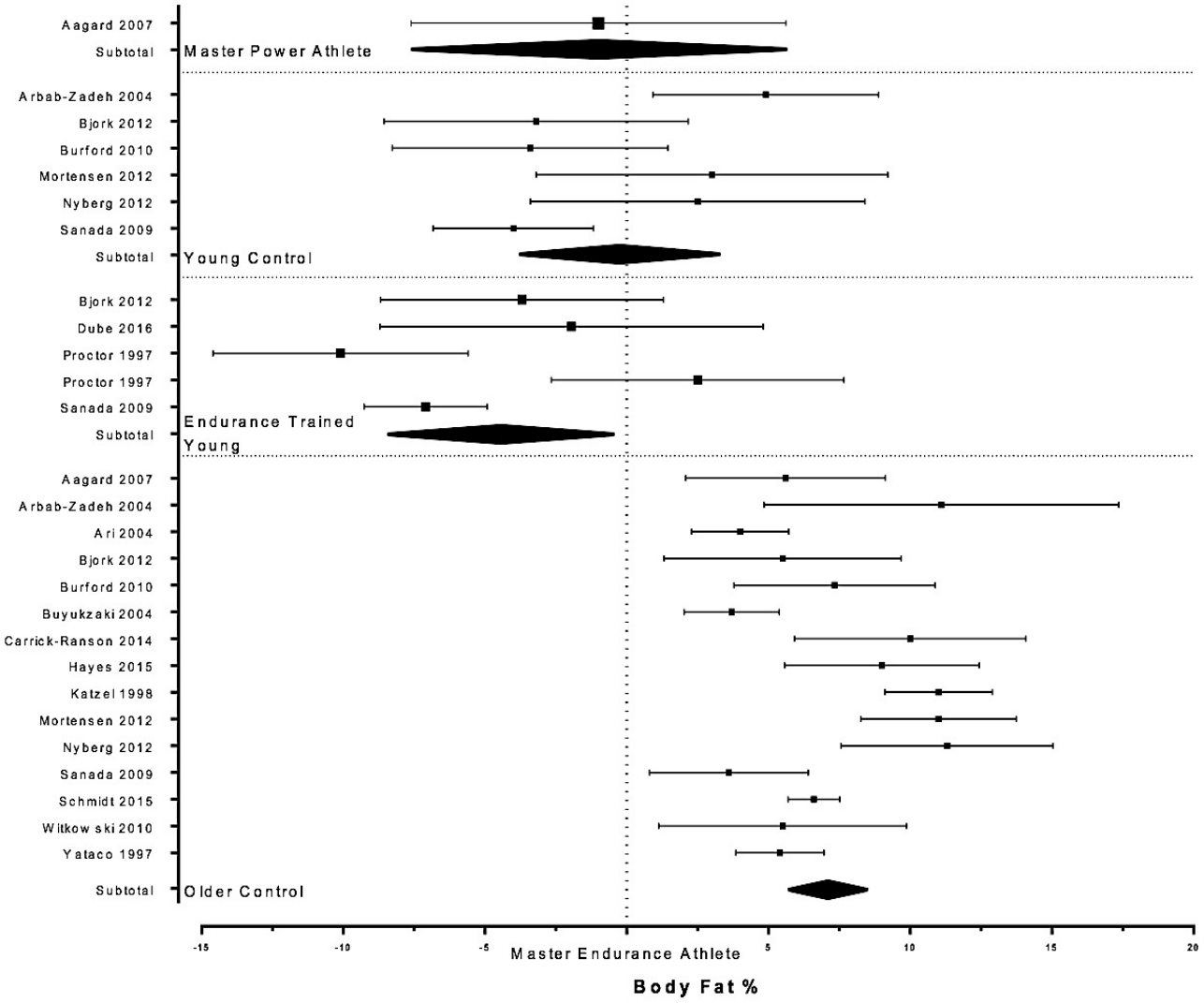

Figure 6. Forest plot of the results from a random-effects meta-analyses shown and mean difference with 95% CI on body fat % in old control, master power athletes, endurance trained young and young control. The vertical dotted line represents the body fat for the master endurance athletes. For each study the square represents the mean difference with the horizontal line indicating the upper and lower limits of the 95% CI. The size of the square indicates the relative weighted contribution of each study to the meta-analyses. The diamond in each section indicates the pooled mean difference for each respective group. Master endurance athletes vs. old control: Heterogeneity: 7.11 (5.70, 8.52) Tau² = 5.36; Chi² = 71.00, df = 14 (P < 0.00001); I² = 80% Test for overall effect: Z = 9.89 (P < 0.00001). Master endurance athletes vs. master power athletes: -1.00 (-7.61, 5.61) Heterogeneity: Not applicable Test for overall effect: Z = 0.30 (P = 0.77). Master endurance athletes vs. young control: -0.22 (-3.72, 3.28) Heterogeneity: Tau² = 13.08; Chi² = 17.30, df = 5 (P = 0.004); I² = 71% Test for overall effect: Z = 0.12 (P = 0.90). Master endurance athletes vs. young endurance trained: -4.44 (-8.44, -0.43) Heterogeneity: Tau² = 15.10; Chi² = 16.76, df = 4 (P = 0.002); I² = 76% Test for overall effect: Z = 2.17 (P = 0.03).

2.4.8. Maximal Oxygen Consumption (VO_{2max})

A meta-analysis was conducted for VO_{2max} for studies that made direct comparisons with master endurance athletes (Figure 7); this allowed for the greatest number of within-study comparisons to be incorporated into the statistical analysis. VO_{2max} was measured in 43 studies (Aagaard et al., 2007a, Anselme et al., 1994, Arbab-Zadeh et al., 2004, Ari et al., 2004, Bhella et al., 2014, Bjork et al., 2012, Buford et al., 2010, Buyukyazi, 2004, Carrick-Ranson et al., 2014, DubÉ et al., 2016, Franzoni et al., 2005, Galetta et al., 2005, Galetta et al., 2006, Hawkins et al., 2001, Hayes et al., 2015, Katzel et al., 1998, Katzel et al., 2001, Mackey et al., 2014, Marcell et al., 2003, Marcell et al., 2014, Matelot et al., 2016, Mikkelsen et al., 2013, Molmen et al., 2012, Mortensen et al., 2012, Mucci et al., 1999, Nyberg et al., 2012, Ojanen et al., 2007, Pollock et al., 2015, Prasad et al., 2007, Prefaut et al., 1994, Proctor and Joyner, 1997, Rivier et al., 1994, Sanada et al., 2009, Schmidt et al., 2015, Shibata and Levine, 2012, Sundstrup et al., 2010, Suominen and Rahkila, 1991, Tarpenning et al., 2004, Thomas et al., 2013, Trappe et al., 2013, Witkowski et al., 2010, Yataco et al., 1997) using treadmill or cycle/rowing ergometer protocols. All studies reported the data relative to bodyweight ($ml.kg^{-1}.min^{-1}$). Forty-nine study groups included master endurance athletes (42.0 ± 6.6 $ml.kg^{-1}.min^{-1}$, n=889), 3 study groups included master power athletes (26.5 ± 2.3 $ml.kg^{-1}.min^{-1}$, n=37), 32 study groups included older controls (27.1 ± 4.3 $ml.kg^{-1}.min^{-1}$, n=602), 13 study groups included young endurance trained individuals (60.0 ± 5.4 $ml.kg^{-1}.min^{-1}$, n=143) and 16 study groups included young controls (43.1 ± 6.8 $ml.kg^{-1}.min^{-1}$, n=221). VO_{2max} was higher in master endurance athletes than older controls ($P<0.0001$) and master power athletes ($P=0.0005$). VO_{2max} was higher in endurance trained young than all other groups ($P < 0.01$). VO_{2max} was higher in young controls than older controls ($P<0.01$) and master

strength/power athletes (P<0.01). No significant differences were observed between young controls and master endurance athletes or between older controls and master strength/power athletes. Young strength/power trained individuals were not included in the meta-analyses as no eligible studies made direct within-study comparisons with the other included groups.

2.4.9. Strength

Maximal voluntary contraction (MVC) was measured in 16 studies (Aagaard et al., 2007a, Couppe et al., 2014, Korhonen et al., 2006, Korhonen et al., 2012, Marcell et al., 2014, Mikkelsen et al., 2013, Mosole et al., 2014, Pollock et al., 2015, Power et al., 2012, Power et al., 2010, Sanada et al., 2009, Sipila and Suominen, 1991, Stenroth et al., 2016, Sundstrup et al., 2010, Suominen and Rahkila, 1991, Tarpenning et al., 2004, Zampieri et al., 2015); 9 studies with 22 study groups were included in a meta-analysis. A meta-analysis was conducted for studies that made direct comparisons with master endurance athletes (Figure 8); to allow for the greatest number of within-study comparisons to be incorporated into the statistical analysis. Due to the diversity of measurement scales/methods of assessment (i.e. knee extension, plantar flexion, elbow flexion and handgrip dynamometry) data are presented as SMD ± 95% CI in Figure 8. MVC was significantly greater in master strength/power athletes (0.60 (0.28, 0.93)) and young healthy controls (0.71 (0.06, 1.36)) than master endurance athletes P<0.01 and P<0.05, respectively. No significant differences were observed in MVC between master endurance athletes when compared with older controls and young endurance trained individuals. Young strength/power trained individuals were not included in the meta-analyses as no eligible studies made direct within-study comparisons with the other included groups.

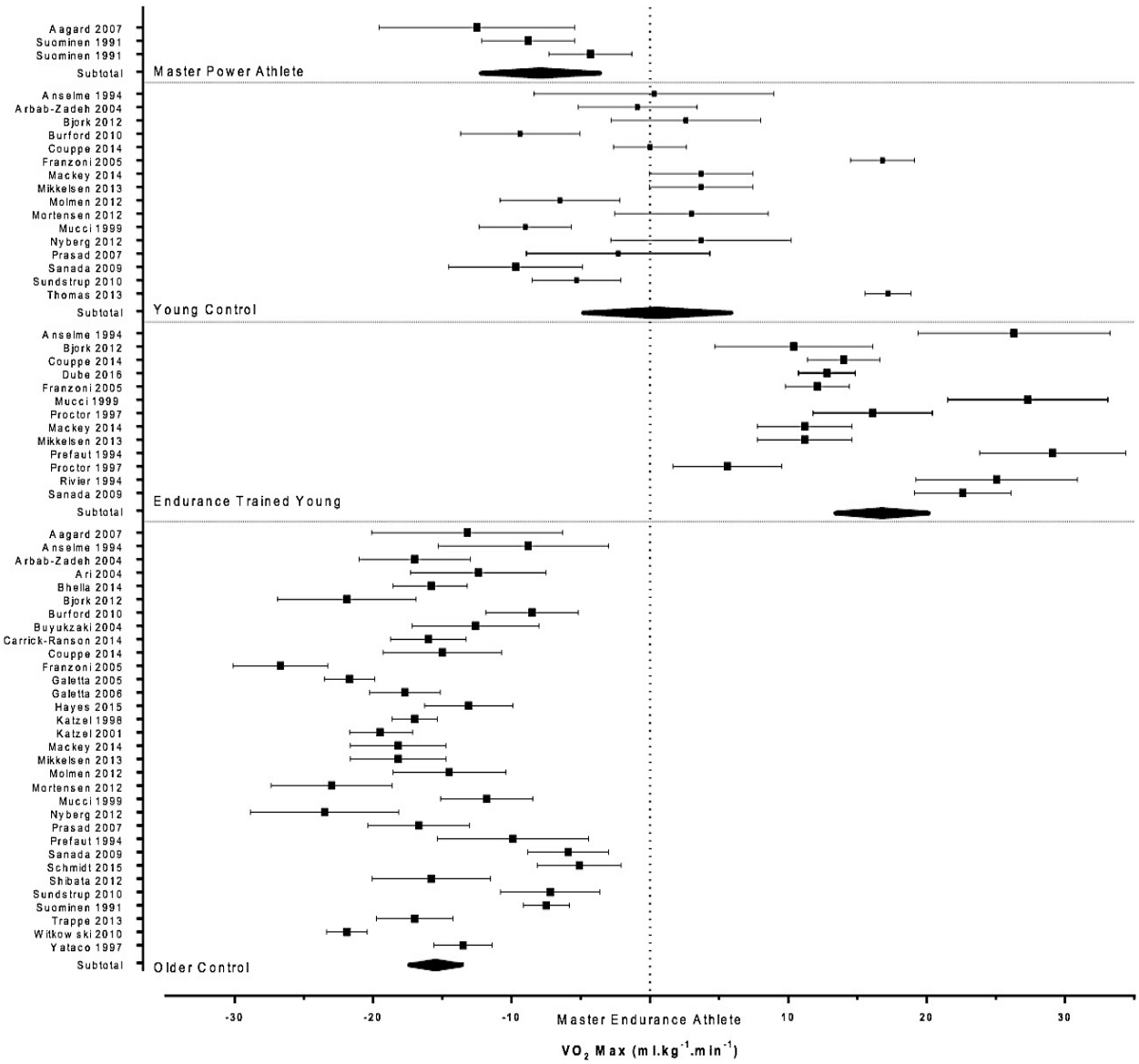

Figure 7. Forest plot of the results from a random-effects meta-analyses shown and mean difference with 95% CI on VO2max (ml.kg-1.min-1) in old control, master power athletes, endurance trained young and young control. The vertical dotted line represents the VO2max for the master endurance athletes. For each study the square represents the mean difference with the horizontal line indicating the upper and lower limits of the 95% CI. The size of the square indicates the relative weighted contribution of each study to the meta-analyses. The diamond in each section indicates the pooled mean difference for each respective group. Master endurance athletes vs. old control: -15.41 (-17.39, -13.43) Heterogeneity: Tau² = 28.97; Chi² = 439.62, df = 31 (P < 0.00001); I² = 93% Test for overall effect: Z = 15.27 (P < 0.00001). Master endurance athletes vs. master power athletes: -7.77 (-12.03, -3.50) Heterogeneity: Tau² = 9.37; Chi² = 6.54, df = 2 (P = 0.04); I² = 69% Test for overall effect: Z = 3.57 (P = 0.0004). Master endurance athletes vs. young control: 0.55 (-4.88, 5.98) Heterogeneity: Tau² = 116.92; Chi² = 547.90, df = 15 (P < 0.00001); I² = 97% Test for overall effect: Z = 0.20 (P = 0.84). Master endurance athletes vs. young endurance trained: 16.79 (13.50, 20.09) Heterogeneity: Tau² = 31.81; Chi² = 128.46, df = 12 (P < 0.00001); I² = 91% Test for overall effect: Z = 9.99 (P < 0.00001).

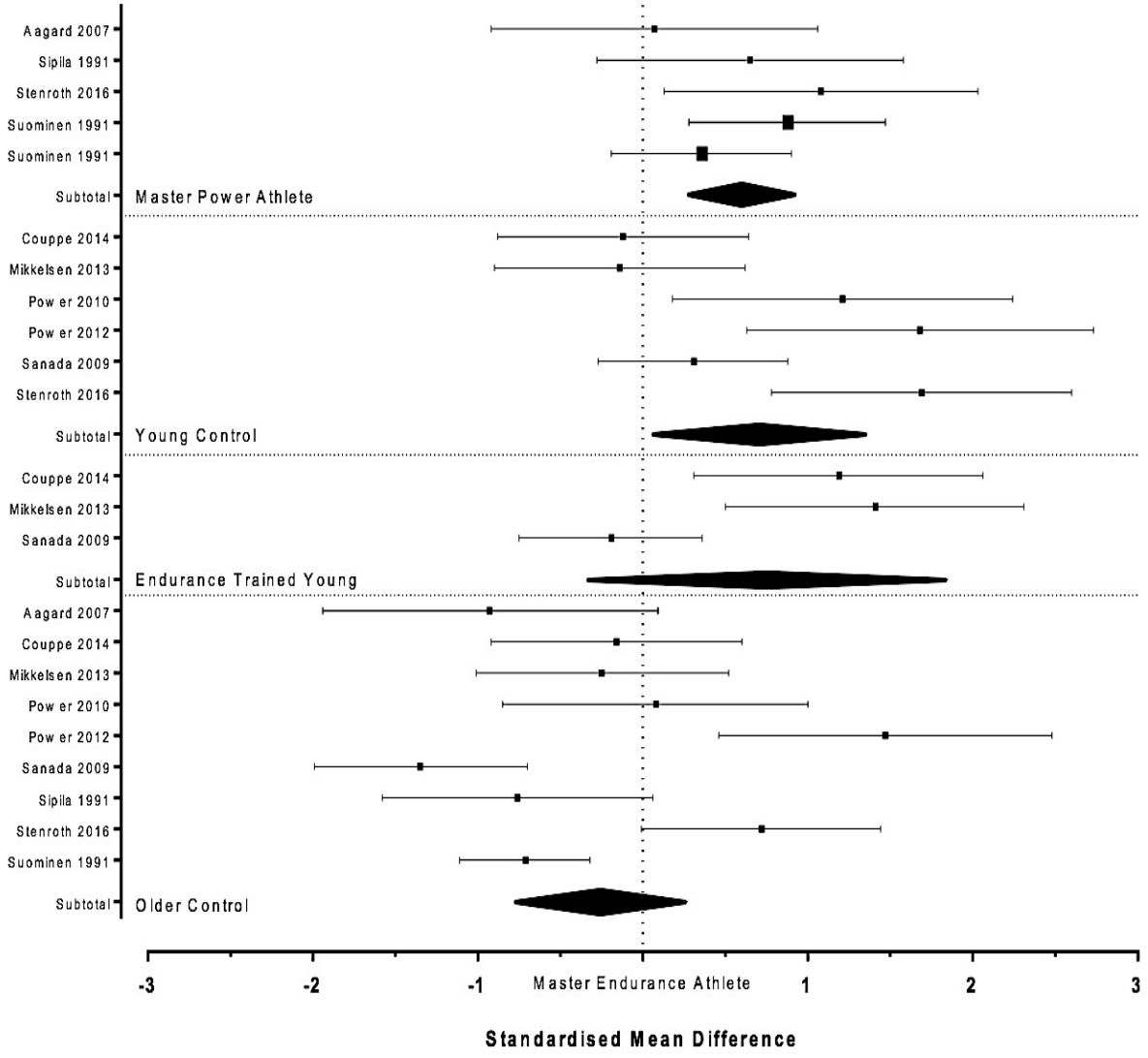

Figure 8. Forest plot of the strength results from random-effects meta-analyses shown presented as standardised mean difference with 95% CI in master power athletes, old control, endurance trained young and young control. The vertical dotted line represents the master endurance athletes. For each study the square represents the standardised mean difference with the horizontal line indicating the upper and lower limits of the 95% CI. The size of the circle indicates the relative weighted contribution of each study to the meta-analyses. The diamond in each section indicates the pooled mean difference for each respective group Master endurance athletes vs. master power athletes: 0.60 [0.28, 0.93] Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 3.68$, $df = 4$ ($P = 0.45$); $I^2 = 0\%$ Test for overall effect: $Z = 3.63$ ($P = 0.0003$). Master endurance athletes vs. old control: -0.25 [-0.77, 0.27] Heterogeneity: $Tau^2 = 0.48$; $Chi^2 = 36.17$, $df = 8$ ($P < 0.0001$); $I^2 = 78\%$ Test for overall effect: $Z = 0.93$ ($P = 0.35$). Master endurance athletes vs. young control: 0.71 [0.06, 1.36] Heterogeneity: $Tau^2 = 0.47$; $Chi^2 = 18.91$, $df = 5$ ($P = 0.002$); $I^2 = 74\%$ Test for overall effect: $Z = 2.13$ ($P = 0.03$). Master endurance athletes vs. young endurance trained: 0.75 [-0.34, 1.85] Heterogeneity: $Tau^2 = 0.78$; $Chi^2 = 12.12$, $df = 2$ ($P = 0.002$); $I^2 = 84\%$ Test for overall effect: $Z = 1.35$ ($P = 0.18$).

2.4.10. Muscle Architecture

Three studies reported muscle architecture outcomes (Korhonen et al., 2006, Ojanen et al., 2007, Sipila and Suominen, 1991); included in those studies were measurements of muscle thickness (3 studies) and fascicle length (1 study). Of the studies that measured muscle thickness, one study (Korhonen et al., 2006) used the midpoint of the *vastus lateralis* in young controls 18-33 y (2.61 ± 0.08 cm, n=16) and master sprinters of 60-69 (2.1 ± 0.09 cm, n=21) and 70-84 y (1.96 ± 0.08 cm, n=20). Another study (Ojanen et al., 2007) summed muscle thickness measurements of the *vastus lateralis* and *vastus intermedius* in master throwers of 65 (3.9 ± 0.7 cm, n=12) and 70 (3.7 ± 0.7 cm, n=9) years compared with older controls, 60 (3.8 ± 0.4 cm, n=10) and 75 (3.1 ± 0.2 cm, n=5), with no differences between the groups. The final study (Sipila and Suominen, 1991) measured muscle thickness of the *rectus femoris,* finding no difference between master strength/power athletes (2.73 ±0.37 cm, n=7), master endurance athletes (2.77 ± 0.39 cm, n=14) and older controls (2.8 ± 0.56 cm, n=11). One study (Korhonen et al., 2006) included measurements of *vastus lateralis* fascicle length which identified no significant differences between the groups of 18-33 year olds (7.91 ± 0.49 cm, n=16), 60-69 (7.99 ± 0.27 cm, n=21) and 70-84 (7.38 ± 0.27 cm, n=20) year old sprint athletes.

2.4.11. Muscle Cross-Sectional Area

Four studies (Couppe et al., 2014, Mikkelsen et al., 2013, Rantalainen et al., 2014, Sipila and Suominen, 1991) reported muscle CSA; two of which used MRI at the mid-thigh level, another used mid-thigh CSA with B-mode ultrasonography and another study used p-QCT to measure mid-tibia muscle CSA. Mid-thigh muscle CSA was reported in two studies, which highlighted muscle size being greater in younger individuals compared

with older individuals and also greater in trained individuals compared with untrained controls; young endurance trained (7859 ± 636 mm^2, n=10), young controls (6792 ± 696 mm^2, n=12), master endurance trained (6481 ±775 mm^2, n=15) and older controls (5504 ± 727 mm^2, n=12) (Couppe et al., 2014, Mikkelsen et al., 2013). Muscle CSA measured using ultrasonography in another study did not identify any differences between master power athletes (5250 ± 1080 mm^2, n=7), master endurance athletes (5270 ± 880 mm^2, n=14) or older controls (4840 ±1110 mm^2, n=11) (Sipila and Suominen, 1991). CSA of the mid-tibia region indicated that exercising individuals have a larger muscle CSA than non-exercising individuals and young individuals have a larger mid-tibia muscle CSA than older controls with no age-group interaction observed (Rantalainen et al., 2014); young power athletes (7140 ± 820 mm^2, n=26), young control (7040 ± 1310 mm^2, n=41), master power athletes (6270 ± 910 mm^2, n=35), older controls (5890 ± 890 mm^2, n=24).

2.4.12. Muscle Fibre Area

In total 9 studies (Aagaard et al., 2007a, Cristea et al., 2008, Korhonen et al., 2006, Larsson et al., 1997, Mackey et al., 2014, Mosole et al., 2014, Sundstrup et al., 2010, Tarpenning et al., 2004, Zampieri et al., 2015) measured muscle fibre morphology (type/distribution), including measures of type I, type II, type IIa, type IIx, type IIax and denervated fibres; using a variety of analytical techniques.

Type 1 fibre area was reported in 5 groups of master endurance athletes (5367 ± 588 μm^2, n=74), 4 groups of master power athletes (4750 ± 1273 μm^2, n=34), 3 groups of older controls (5488 ± 459 μm^2, n=28), 2 groups of young controls (4282 ± 26 μm^2, n=28) and 1 group of young endurance athletes (5550 ± 1337 μm^2, n=10).

Type 2 fibre area was reported in 3 groups of master endurance athletes (4478 ± 347 μm^2, n=55), 1 group of older controls (4149 ± 903 μm^2, n=12) 1 group of young controls (4783 ± 872 μm^2, n=12) and 1 group of young endurance trained (5498 ± 1559 μm^2, n=10).

Type 2a fibre area was reported in 2 groups of master endurance athletes (5043 ± 41 μm^2, n=19), 4 groups of master strength/power athletes (4755 ± 1404 μm^2, n=34), 2 groups of older controls (5411 ± 484 μm^2, n=16) and 1 young control group (4700 ± 560 μm^2, n=16).

Type 2x fibre area was reported in 2 groups of master endurance athletes (3955 ± 88 μm^2, n=19), 4 groups of master strength/power athletes (4019 ± 990 μm^2, n=34), 2 groups of older controls (3794 ± 0 μm^2, n=16) and 1 young control group (3200 ± 2720 μm^2, n=16).

Type 2ax fibre area was reported in 1 young control group (3700 ± 760 μm^2, n=16) and 3 groups of master strength/power athletes (4120 ± 495 μm^2, n=27).

2.4.13. Muscle Fibre Distribution

Type 1 fibre distribution was reported in 7 groups of master endurance athletes (60.8 ± 10.7 %, n=96), 5 groups of master strength/power athletes (48.0 ± 8.0 %, n=59), 6 groups of older controls (49.8 ± 4.2 %, n=45), 4 young control groups (43.5 ± 4.5 %, n=37), and 2 young endurance trained groups (51.5 ± 13.4 %, n=15).

Type 2 fibre distribution was reported in 5 groups of master endurance athletes (38.4 ± 11.3 %, n=77), 3 groups of older controls (50.3 ± 4.0 %, n=27), 2 young control groups (53.5 ± 5.0 %, n=17) and 2 young endurance trained groups (48.6 ± 13.5 %, n=15).

Type 2a fibre distribution was reported in 2 groups of master endurance athletes (32.6 ± 10.7 %, n=19), 5 groups of master strength/power athletes (31.8 ± 3.8 %, n=59), 2 groups of older controls (23.5 ± 0 %, n=16) and 1 young control group (35 ± 12 %, n=16).

Type 2x fibre distribution was reported in 3 groups of master endurance athletes (10.3 ± 2.7 %, n=21), 5 groups of master strength/power athletes trained individuals (10.7 ± 4.1 %, n=59) 3 groups of older controls (22.2 ± 13.1 %, n=18) and 2 young control groups (7.5 ± 6.4 %, n=20).

Type 2ax fibre distribution was reported in 2 groups of master endurance athletes (6.3 ± 8.1 %, n=9), 4 groups of master strength/power athletes (11.6 ± 2.6 %, n=52), 2 groups of older controls (5.9 ± 5.8 %, n=8), 2 young control groups (12.0 ± 00 %, n=20) and 1 groups of young endurance trained individuals (0.5 ± 0.6 %, n=5).

The percentage of denervated fibres, defined as fibre size smaller than 30μm, was reported in 2 groups of master endurance athletes (1.9 ± 0.1 %, n=22), 2 groups of older controls (5.3 ± 1.8 %, n=15) 1 young control group (0.3 ± 0.0 %, n=5) nand1 young endurance trained group (0.4 ± 0.5 %, n=5).

2.5. DISCUSSION

To our knowledge, this is the first systematic review and meta-analyses to focus on the effect of long-term exercise (i.e., endurance or strength/power) on physical function, muscular strength, muscle mass and morphology, to understand whether chronic exercise training preserves these parameters compared with age-matched untrained individuals, as well as younger trained and untrained individuals. There has been considerable debate about the relative contribution of primary and secondary ageing to the deterioration in performance and skeletal muscle parameters and whether or not this deterioration is inevitable in ageing. Our principal findings demonstrate that master endurance athletes are able to completely prevent the decline in VO_{2max} found in age-matched untrained individuals; to values similar to those observed in young untrained individuals. In addition, we demonstrate that master strength/power athletes possess greater strength compared with age-matched master endurance athletes and untrained individuals; comparable with young untrained individuals. Further, all master athletes maintained a similar body fat percentage to untrained young controls, whereas untrained older individuals had a higher body fat percentage than endurance trained young. In the absence of structured exercise training, many older individuals displaying impairments in exercise performance and functional capacity (Tanaka and Seals, 2008), increased fat and reduced lean mass (Evans and Campbell, 1993) and changes to muscle fibre morphology (i.e. fibre atrophy and shifts in fibre sub-type) (Zampieri et al., 2015). Nevertheless, our findings, as well as those of others, demonstrate that older individuals maintain the capacity to adapt to exercise stimuli (Malbut et al., 2002, Newton et al., 2002). The results from this review highlight that regardless of the exercise modality (i.e., endurance or strength/power), chronic exercise training delays the canonical age-related deterioration in physical

function and body composition. The maintenance/continuation of structured exercise training into older age may therefore be seen as the cornerstone to optimal ageing and extension of the health-span in our ageing population.

2.5.1 VO_{2max}

The reduction in function of multiple bodily systems observed with ageing leads to a decline of VO_{2max} at a rate of equivalent to ~10-12% per decade (or ~1% per annum) (Rogers et al., 1990). Cardiorespiratory fitness is independently associated with all-cause mortality regardless of age, smoking status, body composition, and other risk factors (Lee et al., 2011, Lee et al., 2010) and thus a high VO_{2max} may be protective against premature mortality. Meta-analysis comparisons revealed a 57% (15.4 $ml \cdot kg^{-1} \cdot min^{-1}$) greater VO_{2max} in master endurance athletes compared with age-matched untrained individuals. Furthermore, master endurance athletes exhibited a greater VO_{2max} (7.8 $ml \cdot kg^{-1} \cdot min^{-1}$) than master strength/power athletes. Collectively, these findings highlight that age-related declines in VO_{2max} can be prevented by chronic exercise in an exercise mode-specific manner.

The reduction in VO_{2max} reported in master athletes appear to begin at the same age and decline at a similar (Faulkner et al., 2008, Wilson and Tanaka, 2000), if not greater ((Tanaka et al., 1997, Fitzgerald et al., 1997, Eskurza et al., 2002)), rate compared with age-matched untrained individuals; providing evidence that despite continuous endurance exercise training there is an unavoidable decline of aerobic capacity. It is proposed that as age progresses, master athletes undergo a greater alteration to their activity status (e.g. training intensity and volume) (Eskurza et al., 2002, Fitzgerald et al., 1997), which may underpin the accelerated rate of decline in VO_{2max} observed (Eskurza et al., 2002, Tanaka

et al., 1997). However, the studies included in the current review highlighted no observable difference in training frequency (Aagaard et al., 2007a, Bhella et al., 2014, Buyukyazi, 2004, Carrick-Ranson et al., 2014, Couppe et al., 2014, DubÉ et al., 2016, Franzoni et al., 2005, Galetta et al., 2005, Galetta et al., 2006, Korhonen et al., 2006, Korhonen et al., 2012, Larsson et al., 1997, Marcell et al., 2003, Marcell et al., 2014, Molmen et al., 2012, Ojanen et al., 2007, Power et al., 2010, Sallinen et al., 2008, Sanada et al., 2009, Sipila and Suominen, 1991, Sundstrup et al., 2010, Witkowski et al., 2010, Yataco et al., 1997, Zampieri et al., 2015), distance (Arbab-Zadeh et al., 2004, Couppe et al., 2014, Hawkins et al., 2001, Mackey et al., 2014, Marcell et al., 2003, Marcell et al., 2014, Mikkelsen et al., 2013, Power et al., 2010, Shibata and Levine, 2012, Stenroth et al., 2016, Suominen and Rahkila, 1991, Tarpenning et al., 2004, Thomas et al., 2013, Witkowski et al., 2010) and session duration (Ari et al., 2004, Bjork et al., 2012, Buford et al., 2010, Buyukyazi, 2004, Carrick-Ranson et al., 2014, Couppe et al., 2014, Korhonen et al., 2006, Korhonen et al., 2012, Matelot et al., 2016, Mortensen et al., 2012, Mosole et al., 2014, Mucci et al., 1999, Nyberg et al., 2012, Proctor and Joyner, 1997, Rantalainen et al., 2014, Rivier et al., 1994, Sanada et al., 2009, Schmidt et al., 2015, Stenroth et al., 2016, Suominen and Rahkila, 1991, Trappe et al., 2013, Zampieri et al., 2015) between younger and older endurance athletes; although alterations to training intensity cannot be excluded as none were reported. VO_{2max} values included in this meta-analysis were ~32% lower in master endurance athletes (42 ± 6.6 $ml \cdot kg^{-1} \cdot min^{-}1$) than young endurance trained individuals (62.0 ± 5.4 $ml \cdot kg^{-1} \cdot min^{-}1$), which is supported by recent research demonstrating an inescapable reduction in VO_{2max} in master endurance athletes, despite continuous levels of endurance exercise training (Everman et al., 2018). It is beyond the scope of this review to identify the relative rate of decline in VO_{2max} as no longitudinal

studies were incorporated. Regardless of the rate at which VO_{2max} declines with age, the maintenance of endurance-type exercise and thus cardiorespiratory fitness are paramount to offset age-associated health decrements. The greater VO_{2peak} in these highly trained master athletes may therefore allow larger reductions to occur before reduced physical capacity and functional independence is compromised, compared with age-matched untrained individuals.

Although not established in the current review, it is generally acknowledged that reduced physical activity is a key contributor towards the development of disease, disability and physical dysfunction (Booth et al., 2012). Continuation of exercise training may protect against the deterioration of health status and age-associated co-morbidities, by commencing the decline in bodily function from an elevated VO_{2peak} the ageing process reflects inherent ageing uncompromised by inactivity (Harridge and Lazarus, 2017). From a mechanistic perspective, the parameters that determine VO_{2max} (cardiac output and arterio-venous difference) are known to decrease with advancing age; inclusive of master athletes (Tanaka and Seals, 2008). The relative contribution of each parameter is unclear, though the reduction of VO_{2max} observed in master endurance athletes may be due to maintenance of stroke volume and O_2 extraction, as they do not appear to decrease by the same magnitude as heart rate with age (Pollock et al., 1987). Consistent with this, maximal heart rate has been shown to decrease at approximately 0.7 $beat.min^{-1}.year^{-1}$, and this is similar regardless of sex or physical activity status (Rodeheffer et al., 1984). In addition, peripheral factors influencing the arterio-venous difference proposed to contribute to the differences in VO_{2max} between older, young, trained and control groups, include the amount of fat-free and fat mass (Tanaka and Seals, 2008), which have been shown to decrease and increase, respectively, with advancing age (Janssen et al., 2000, Kuk et al.,

2009). However, the results from the present review demonstrated no discernible differences between groups in the amount of fat-free mass for master endurance athletes, age-matched untrained individuals, young untrained individuals and young endurance trained individuals. Wroblewski and colleagues (2011) demonstrated that chronic endurance exercise is able to preserve thigh muscle CSA in master athletes (Wroblewski et al., 2011). From our data, it is perhaps surprising that the older untrained individuals did not display a marked reduction in fat-free mass with advancing age. However, it has previously been shown that VO_{2max} remains lower in older trained individuals compared with young trained individuals, despite corrections for muscle mass (Proctor and Joyner, 1997). Therefore, it is more likely that reductions in capillary density, mitochondrial function and enzyme activity (Coggan et al., 1990) are responsible for the lower VO_{2max} values observed in master endurance athlete and older controls.

2.5.2. Strength

Muscle strength and power are ~40 and ~80 % lower in older vs. younger individuals (Thom et al., 2007). At the top level of competition, this is reflected by decreases in sprint times, jumping and throwing distances (Gava et al., 2015). In terms of the general population, the functional significance of this decline impacts individuals' ability to maintain ADL such as stair climbing, rising from a chair or opening a jar (Hairi et al., 2010). Moreover, low levels of muscular strength are strongly associated with an elevated risk of functional impairment (Cruz-Jentoft et al., 2010) and all-cause mortality (Ruiz et al., 2008, Ortega et al., 2012). The current meta-analyses (Figure 8) revealed that chronic endurance exercise does not prevent age-related declines in muscular strength. However, strength was preserved in master strength/power athletes to a level comparable

with young untrained individuals, again suggesting that preservation of physical function into older age is specific to the mode of exercise performed.

It should be noted that assessment of strength within the studies included in the meta-analyses was achieved through a variety of methods; knee extension MVC, unilateral (Zampieri et al., 2015, Sundstrup et al., 2010, Sipila and Suominen, 1991, Mosole et al., 2014, Pollock et al., 2015, Mikkelsen et al., 2013, Marcell et al., 2014, Korhonen et al., 2012, Couppe et al., 2014, Aagaard et al., 2007a) and bilateral (Korhonen et al., 2006), plantar flexion MVC (Stenroth et al., 2016), dorsiflexion MVC (Power et al., 2010), elbow flexion MVC (Power et al., 2012) and handgrip strength (Suominen and Rahkila, 1991, Sanada et al., 2009)). In addition, the present review lacked a young strength/power trained group for comparison, as no studies incorporating this population met the inclusion criteria for the review. Therefore, we cannot draw firm conclusions on whether master strength/power athletes were able to maintain strength similar to that of young strength/power trained athletes. However, it is generally accepted that, similar to VO_{2max}, there is an inevitable decline in muscle strength/power with ageing (Goodpaster et al., 2006, Rittweger et al., 2009), evidenced longitudinally in the performance decrements of master power vs. young power trained athletes (Gava et al., 2015). This suggests that the relative rate of decline in muscle strength is similar between trained individuals and age-matched controls. However, the higher absolute levels of strength provide strength-trained individuals with a much greater strength reserve, thus delaying the onset of impaired function and loss of independence.

2.5.4. Muscle Mass and Morphology

One mechanism responsible for age-related strength loss is the decline in muscle mass (sarcopenia) (Narici and Maffulli, 2010). In young healthy individuals, muscle mass is generally maintained until the 5th decade of life, after which loss of mass progresses at a rate of ~0.5-1% per year (Mitchell et al., 2012b); and is exacerbated by protracted disuse events (Alkner and Tesch, 2004). Thus, by the age of 80 years an individual may have lost ~30-40% of peak muscle mass (Janssen et al., 2000). Due to the central role of skeletal muscle in physical function, basal metabolism and nutrient deposition, muscle loss can have significant health implications. The results from the current review suggest no age or training-related differences in muscle mass between groups (master endurance, older untrained, young untrained and young endurance trained individuals). This may be due to a paucity of studies that incorporated muscle mass assessment, or that most of the included studies used techniques (BIA, DXA, hydrostatic weighing) with lower precision and sensitivity in cross-sectional comparisons (MacDonald et al., 2011). The absence of any difference in muscle mass between groups is perhaps surprising given that the older individuals included in the current review were over 60 years and, therefore, beyond the age-range where sarcopenia or pre-sarcopenia (loss of muscle mass prior to any functional decline (Pereira et al., 2015)) may begin to manifest. The lack of consistency between studies highlights that further investigation into age and training-related differences in muscle mass is warranted.

It has been shown muscle mass/volume is associated with muscular strength in young and older men and women (Young et al., 1984, Strasser et al., 2013), however there is a clear dissociation in the rate at which each declines, with strength loss progressing at a greater rate than muscle loss (Delmonico et al., 2009, Frontera et al., 2000).

Concomitant with the changes in muscle mass, alterations to the neuromuscular system may also influence muscle force generating capacity, including reductions in the number of motor units, increased size and stimulation thresholds of existing motor units as a result of cyclical denervation and re-innervation, and increased instability of transmission at the neuromuscular junction (Piasecki et al., 2016c, Piasecki et al., 2016b). Two studies (Mosole et al., 2014, Zampieri et al., 2015) identified the presence of greater numbers of denervated fibres in the muscles of the older controls, when compared with young and older untrained individuals. This lends credence to the notion that chronic exercise training, both endurance and resistance, is able to preserve the neural component of muscular contraction. There is evidence to suggest that the muscle quality (i.e., the force per unit of muscle) is more important than muscle mass per se and more closely associated with mortality (Newman et al., 2006). Muscle quality diminishes with age due to changes in architecture (Strasser et al., 2013), fibre type morphology (Lexell, 1995) fat and non-contractile tissue infiltration (Kent-Braun et al., 2000), satellite cells and myonuclei (Kadi et al., 2004). Although it was not possible to draw firm conclusions on the impact of chronic exercise training on muscle mass and quality, it is clear that chronic strength/power exercise can prevent age-associated strength decrements.

Ageing is accompanied by a reduction in the total number of muscle fibres, but more critically the cross-sectional area and proportion of Type II fibres (Lexell, 1995). However, due to the paucity of studies reporting muscle cross sectional area (Couppe et al., 2014, Mikkelsen et al., 2013, Rantalainen et al., 2014, Sipila and Suominen, 1991), muscle architecture (Korhonen et al., 2006, Ojanen et al., 2007, Sipila and Suominen, 1991) and fibre morphology (Aagaard et al., 2007a, Cristea et al., 2008, Korhonen et al., 2006, Larsson et al., 1997, Mackey et al., 2014, Mosole et al., 2014, Sundstrup et al.,

2010, Tarpenning et al., 2004, Zampieri et al., 2015), it is challenging to elucidate their relative contribution to the observed loss or preservation of muscle mass and strength between master athletes and age-matched untrained individuals. Nevertheless, previous studies have highlighted an association between muscle architecture and performance (Kumagai et al., 2000), and that muscle architecture may differ across sporting disciplines (Abe et al., 2000). Upon further investigation, differences in muscle CSA, thickness and morphology may be an artefact of differing analytical methods (i.e. MRI, CT, ultrasound) at divergent anatomical sites (i.e. thigh vs. mid-tibia). Though not statistically analysed in the current review, individual studies have shown that master strength/power athletes maintain greater fibre cross-sectional area compared with untrained older individuals (Aagaard et al., 2007a, Korhonen et al., 2006). In addition to fibre atrophy, ageing increases the intramuscular infiltration of fat and connective tissue (Taaffe et al., 2009), impairing contractile properties (Lexell, 1995). Studies have revealed conflicting findings with respect to muscle fibre distribution between young and older endurance trained individuals (Mackey et al., 2014, Mosole et al., 2014, Sundstrup et al., 2010, Zampieri et al., 2015), which are likely underpinned by differences in the specific mode of exercise training and analytical methods used (Summary data for studies that measured these outcomes demonstrate large heterogeneity; presented in Table 3).

2.5.5. Body Fat Mass

Age-related alterations in body composition, specifically the increase in adiposity, likely contributes to impairments in muscle strength and physical function, increases dependence on support services and the likelihood of institutionalised care (Guralnik et al., 1996) and ultimately presents a significant risk factor for mortality (Kuk et al., 2006). The

current meta-analyses (Figure 6) illustrated that master endurance athletes had a significantly lower body fat percentage compared with age-matched controls. Body fat percentage was similar between master endurance, master strength/power and untrained young individuals, whereas young endurance trained individuals had a significantly lower body fat percentage than master endurance trained individuals. These data indicate that chronic exercise, endurance or strength/power oriented, can attenuate the increase in body fat with ageing. It has been shown that higher levels of physical activity are preventative for body fat mass gain (Ekelund et al., 2011). Further, the reduction in physical activity that occurs during ageing (from retirement, family commitments, and a lack of other preoccupations) is a critical driver of increasing body fat mass (Booth et al., 2012). Unfortunately, few studies included in this review provided data for absolute values of fat and fat-free mass, making it difficult to draw conclusions about effect that chronic exercise training has on these parameters. Interestingly, it has previously been shown that obese individuals possess higher levels of fat-free mass compared with healthy older individuals (Murton et al., 2015), suggesting a minor protective role of obesity against sarcopenia. Nevertheless, we posit that muscle quality, and not quantity *per se*, plays a more important role in the maintenance of whole-body metabolic health (Smeuninx et al., 2017).

2.6 Conclusions and Practical applications

The aim of this systematic review and meta-analyses was to determine whether, and to what extent, chronic exercise preserves some of the functional and biological decrements attributed to ageing. This review demonstrates that chronic exercise may delay

age-associated decrements in physical function in an exercise-mode specific manner and also protects against unfavourable changes in body composition.

Current physical activity guidelines for older adults suggest 150 minutes per week of moderate-to-vigorous physical activity per week, unless chronic conditions prevent them doing so (American College of Sports, 2009). Guidelines also recommend that older adults incorporate some form resistive based exercise at least twice a week. However, the prevalence of functional impairment and reduced independence in older age suggests many older individuals are not meeting these criteria. The master athlete cohorts included in this review provide evidence that canonical age-related impairments in physical function, cardiorespiratory fitness, muscular strength and body composition can be delayed through chronic training, in an exercise mode-specific manner. The continuation of endurance type exercise to maintain high levels of cardiorespiratory fitness and strength/power training to preserve muscular strength offers a viable strategy to extend the health-span of older individuals, compressing the area under the morbidity curve and allowing maintenance of functional independence and good quality of life. It is apparent that few high quality studies have been conducted in unique master athlete cohorts, and that further research studies encompassing a comprehensive selection of sophisticated measurement tools need to be conducted in order to fully characterise specific benefits of strength/power training and endurance training. Finally, the inclusion of only 268 females in this review reflects a preponderance of males included in the study of master athletes. This disparity warrants further investigation, as it has been documented that men and women may undergo somewhat divergent deterioration in skeletal muscle mass and strength with advancing age, perhaps due to reductions in sex-specific hormones (Smith et al., 2008, Kim et al., 2016b, Hansen and Kjaer, 2014). As such, the development of sex-

specific exercise training interventions, or manipulation of certain training variables, may be an important consideration.

2.7 Tables

Author, Year	Fat-Free Mass	Fat Mass	Body Fat	VO_{2max}	Strength	Muscle Architecture	Muscle CSA	Muscle Morphology
(Aagaard et al., 2007)	–	–	Skinfold	Cycle Ergometer	Knee Extension MVC (70° knee angle)	–	–	Muscle Fibre Area, distribution, distribution by area (myofibrillar ATPase staining (Type I, IIa, IIx)
(Anselme et al., 1994)	–	–	–	Cycle Ergometer (30W 3mins, ↑ 30W.min^{-1})	–	–	–	–
(Arbab-Zadeh et al., 2004)	–	–	Hydrostatic Weighing	Treadmill (modified Astrand-Saltin)	–	–	–	–
(Ari et al., 2004)	–	–	No Details	Cycle Ergometer (Astrand)	–	–	–	–
(Bhella et al., 2014)	–	–	–	Treadmill (modified Astrand-Saltin)	–	–	–	–
(Bjork et al., 2012)	–	–	7-Site Skinfold	Treadmill (Constant speed, 2% ↑ grad every 2mins)	–	–	–	–
(Buford et al., 2010)	–	–	DXA	Treadmill	–	–	–	–
(Buyukyazi, 2004)	–	–	4-Site Skinfold	Cycle Ergometer (Astrand-Ryhming Sub-max)	–	–	–	–
(Carrick-Ranson et al., 2014a)	Hydrostatic Weighing	–	Hydrostatic Weighing	Treadmill & Upright cycle	–	–	–	–
(Couppe et al., 2014)	–	–	–	Cycle Ergometer (50/75W start, 25W ↑ .min^{-1})	Knee Extension MVC (knee & hip angle 90°, 10 s ramped contraction)	–	Anatomical CSA measured 20 cm proximal to tibia plateau (mid-thigh level) by magnetic resonance imaging (MRI)	–
(Cristea et al., 2008)	–	–	Bioelectrical Impedance	–	–	–	–	Fibre area & distribution (myofibrillar ATPase staining (Type I, IIa, IIx, IIax))
(DubÉ et al., 2016)	DXA Scan	DXA Scan	DXA Scan	Cycle Ergometer (50/75/10W 2mins, 25/50W ↑ every 2 mins)	–	–	–	–
(Franzoni et al., 2005)	–	–	–	Cycle Ergometer	–	–	–	–
((Galetta et al., 2005)	–	–	–	Cycle Ergometer	–	–	–	–
(Galetta et al., 2006)	–	–	–	Cycle Ergometer	–	–	–	–
(Hawkins et al., 2001)	Hydrostatic Weighing	–	Hydrostatic Weighing	Treadmill (Modified Balke)	–	–	–	–
(Hayes et al., 2015)	Bioelectrical Impedance	–	Bioelectrical Impedance	Cycle Ergometer (Ramp)	–	–	–	–
(Katzel et al., 1998)	–	–	Hydrostatic Weighing	Treadmill (Modified Balke)	–	–	–	–
(Katzel et al., 2001)	–	–	–	Treadmill (Modified Balke)	–		–	–
(Korhonen et al., 2006)	–	–	Bioelectrical Impedance	–	Bilateral Isometric MVC (107° knee & 110° hip angle)	Muscle thickness & pennation angle (50% *Vastus lateralis*)	–	Fibre area & distribution (myofibrillar ATPase staining (Type I, IIa, IIx, IIax))
(Korhonen et al., 2012)	–	–	Bioelectrical Impedance	–	Unilateral Isometric MVC (90° knee & 110° hip angle)	–	–	–
(Larsson et al., 1997)	–	–	–	–	–	–	–	Fibre distribution (myofibrillar ATPase staining (Type I, IIa, IIx, IIax))

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Author, Year	Fat-Free Mass	Fat Mass	Body Fat	VO_{2max}	Strength	Muscle Architecture	Muscle CSA	Muscle Morphology
(Mackey et al., 2014)	–	–	–	Cycle Ergometer	–	–	–	Fibre Area & distribution (primary/secondary antibodies (Type I & II))
(Marcell et al., 2003)	Hydrostatic Weighing		Hydrostatic Weighing	Treadmill (Modified Balke)	–	–	–	–
(Marcell et al., 2014)	Hydrostatic Weighing	–	Hydrostatic Weighing	Treadmill (Modified Balke)	Unilateral Isometric MVC (60° knee flexion)	–	–	–
(Matelot et al., 2016)	–	–	No Details	Cycle Ergometer	–	–	–	–
(Mikkelsen et al., 2013)	–	–	–	Cycle Ergometer (50/75W start, ↑ 25W.min^{-1})	Knee Extension MVC (knee & hip angle 90°, 10 s ramped contraction)	–	Anatomical CSA measured 20 cm proximal to tibia plateau (mid-thigh level) by magnetic resonance imaging (MRI)	–
(Molmen et al., 2012)	–	–	–	Treadmill (Gradient 10% with ↑ speed every min)	–	–	–	–
(Mortensen et al., 2012)	–	–	No Details	Cycle Ergometer		–	–	–
(Mosole et al., 2014)	–	–	–	–	Knee Extension MVC (Relative)	–	–	Fibre distribution (H + E & primary secondary antibodies (type I, II, co-expressing, denervated))
(Mucci et al., 1999)	–	–	–	Cycle Ergometer (30 W 3 mins, ↑30 W min^{-1})	–	–	–	–
(Nyberg et al., 2012)	–	–	No Details	Cycle Ergometer	–	–	–	–
(Ojanen et al., 2007)	–	–	4-Site Skinfold/ Bioelectrical Impedance	–	–	Muscle thickness (*Vastus lateralis* + *Vastus intermedius*, lower 1/3 between greater trochanter & lateral joint line of knee)	–	–
(Pollock et al., 2015)	DXA Scan	–	DXA Scan	Cycle Ergometer (50 W 3 mins, ↑ 1–2 W every 3–5 s)	Knee Extension MVC (90° Knee angle)	–	–	–
(Power et al., 2012)	–	–	–	–	Bicep Brachii MVC (110° Elbow flexion)	–	–	–
(Power et al., 2010a)	–	–	–	–	Tibialis Anterior MVC	–	–	–
(Prasad et al., 2007)	–	–	–	No details	–	–	–	–
(Prefaut et al., 1994)	–	–	–	Cycle Ergometer (30 W 3 mins, ↑ 30 W min^{-1})	–	–	–	–
(Proctor and Joyner, 1997)	DXA Scan	–	DXA Scan	Treadmill (2% ↑ grad every other min)	–	–	–	–
(Rantalainen et al., 2014)	–	–	–	–	–	–	Peripheral quantitative computed tomography (*p*-QCT)	–
(Rivier et al., 1994)	–	–	–	Cycle Ergometer (30 W 3 mins, ↑ 30 W min^{-1})	–	–	–	–
(Sallinen et al., 2008)			4-Site Skinfold	–	–	–	–	–
(Sanada et al., 2009)	DXA Scan	–	DXA Scan	Cycle Ergometer (90 W, ↑ 30 W min^{-1}) Rowing Ergometer 100 W start, 50 W ↑.min^{-1})	Handheld dynamometer	–	–	–

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Author, Year	Fat-Free Mass	Fat Mass	Body Fat	VO_{2max}	Strength	Muscle Architecture	Muscle CSA	Muscle Morphology
(Schmidt et al., 2015)	–	DXA Scan	DXA Scan	Cycle Ergometer (40 W start, ↑ 20 W every 2 mins)	–	–	–	–
(Shibata and Levine, 2012)	–	–	–	No details	–			–
(Sipila and Suominen, 1991)	–	–	–	–	Knee extension MVC (60° Knee angle)	Muscle thickness (no details on site of measurement)	Ultrasound Scanning (midpoint between greater trochanter and knee joint line)	–
(Stenroth et al., 2016)	–	–	–	–	Plantar Flexion MVC (ankle 90°, knee full extended, hip 60° angle)	–	–	–
(Sundstrup et al., 2010)	–	–	–	No details	Knee Extension MVC (70° Knee angle)	–	–	Fibre area, distribution, distribution by area (myofibrillar ATPase staining (Type I, IIa, IIx, IIax))
(Suominen and Rahkila, 1991)	–	–	–	Cycle Ergometer (90W start, ↑30 W every 2 mins)	Handheld dynamometer	–	–	–
(Tarpenning et al., 2004b)	Hydrostatic Weighing	–	Hydrostatic Weighing	Treadmill (2.5mph start, ↑ 0.5 mph and 2% grade every 2 mins)	–	–	–	Fibre area & distribution (myofibrillar ATPase staining (Type I & II))
(Thomas et al., 2013)	–	–	–	Treadmill (modified Astrand-Saltin)	–	–	–	–
(Trappe et al., 2013)	DXA Scan	–	DXA Scan	Cycle Ergometer (Trained: 50 W start ↑15 W min^{-1}, 20 W start ↑10 w min^{-1})	–	–	–	–
(Witkowski et al., 2010)	–	–	DXA Scan	Cycle Ergometer (50W start ↑15W.min^{-1})	–	–	–	–
(Yataco et al., 1997)	Hydrostatic Weighing	Hydrostatic Weighing	Hydrostatic Weighing	Treadmill (Modified Balke)	–	–	–	–
(Zampieri et al., 2015)	–	–	–	–	Knee extension MVC	–	–	Fibre diameter & distribution (H + E, myofibrillar ATPase staining (Type I & II))

Table 1 Included studies and associated measures. DXA, Dual energy x-ray absorptiometry; H + E, Hematoxylin and eosin; MVC, maximal voluntary contraction; W, Watts.

Author, Year	Participant Groups	Number (n)	Sex (M/F)	Age (Years)	Height (m)	Weight (kg)	BMI (Kg.m^2)	Training Experience (years)	Type of Training
(Aagaard et al., 2007)	Older Control	8	M	70.5 (2.8)	1.75 (0.06)	82.9 (6.2)	27.1	–	–
	Master Endurance Athlete	9	M	71.9 (4.3)	1.74 (0.07)	76.0 (8.3)	25.2	> 50	Running/Cycling
	Master power athletes	7	M	73.9 (2.2)	1.75 (0.04)	78.7 (20.1)	25.7	> 50	Sprinting/Shotput/high- and long-jump
(Anselme et al., 1994)	Young Endurance	7	M	22.2 (3.3)	1.80 (0.05)	68.6 (2.9)	21.3	5 (3.7)	Triathletes
	Master Endurance Athlete	7	M	66.2 (7.8)	1.69 (0.06)	75.4 (5.9)	26.5	30 (13.2)	Cyclists
	Young Control	7	M	23.4 (5.2)	1.75 (0.06)	67.2 (12.0)	22.0	–	Habitually active
	Older Control	7	M	67.6 (7.3)	1.67 (0.04)	67.6 (2.8)	24.1	–	Habitually active
(Arbab-Zadeh et al., 2004)	Older Control	12	M = 6 F = 6	69.8 (3.0)	1.68 (0.10)	73.3 (10.6)	25.9	–	–
	Young Control	14	M = 7 F = 7	28.9 (5.0)	1.74 (0.06)	71.2 (4.4)	23.6	–	–
	Master Endurance Athlete	12	M = 6 F = 6	67.8 (3.0)	1.70 (0.11)	64.6 (13.5)	22.4	23 (8)	Running/Cycling/Swimming
(Ari et al., 2004)	Master Endurance Athlete	10	M	68.0 (6.0)	1.67 (0.08)	71.0 (3.0)	25.5	41 (8)	Aerobic
	Older Control	11	M	65.0 (5.0)	1.69 (0.05)	81.0 (11.0)	28.4	–	–
(Bhella et al., 2014)	Older Control	27	M = 15 F = 12	68.8 (5.1)	1.70 (0.10)	74.7 (11.2)	26.0	–	–
	Master Endurance Athlete	25	M = 17 F = 8	67.8 (2.9)	1.71 (0.10)	65.6 (12.1)	22.4	25	Running/Cycling/Swimming
(Bjork et al., 2012)	Young Endurance	7	M	25.0 (5.3)	1.83 (0.26)	81.1 (12.7)	24.4	> 3	Moderate-high intensity
	Young Control	8	M	25 (2.8)	1.81 (0.28)	77.9 (19.0)	23.6	–	–
	Master Endurance Athlete	12	M	62 (6.9)	1.76 (0.35)	70.7 (10.4)	22.9	> 30	Moderate-high intensity
	Older Control	11	M	64 (6.6)	1.75 (0.33)	74.7 (8.0)	24.3	–	–
(Buford et al., 2010)	Young Control	14	M	21.4 (3.8)	1.77 (0.06)	79.6 (17.1)	25.4	–	–
	Older Control	13	M	63.9 (6.6)	1.74 (0.06)	93.7 (15.3)	30.9	–	–
	Master Endurance Athlete	14	M	60.7 (5.5)	1.76 (0.05)	84.2 (7.2)	27.1	23.08 (12.6)	Running/Jogging/Basketball/Resistance
(Buyukyazi, 2004)	Master Endurance Athlete	11	M	67.1 (6.0)	1.67 (0.08)	70.9 (3.2)	25.5	38.8 (18.5)	Middle/Long-distance Runners
	Older Control	11	M	64.9 (4.6)	1.69 (0.06)	81.6 (11.4)	28.3	–	–
(Carrick-Ranson et al., 2014a)	Older Control	27	M = 15 F = 12	69 (5.0)	1.69 (0.10)	75.0 (11.0)	26.3	–	–
	Master Endurance Athlete	25	M = 17 F = 8	68 (3.0)	1.71 (0.10)	66.0 (12.0)	22.6	25	Running/Cycling/Swimming
(Couppe et al., 2014)	Young Endurance	10	M	26.0 (4.0)	1.79 (0.04)	73.0 (6.0)	23.0	6 (3.16)	Endurance running
	Young Control	12	M	24.0 (3.0)	1.78 (0.06)	70.0 (8.0)	22.0	–	–
	Master Endurance Athlete	15	M	64.0 (4.0)	1.76 (0.05)	71.0 (6.0)	23.0	28 (7.75)	Endurance running
	Older Control	12	M	66.0 (4.0)	1.75 (0.04)	75.0 (4.0)	25.0	–	–
(Cristea et al., 2008)	Master power athletes	7	M	66.0 (7.9)	1.73 (0.05)	71.3 (6.6)	23.8	32 (18.5)	Sprint/Strength
	Master power athletes	4	M	71.0 (10.0)	1.71 (0.04)	69.6 (7.2)	23.8	24 (8)	Sprint Strength
(DubÉ et al., 2016)	Young Endurance	14	M = 7 F = 7	27.8 (4.9)	–	65.7 (10.8)	22.12	5–13	Non-competitive recreational exercise
	Master Endurance Athlete	13	M = 9 F = 4	64.8 (4.9)	–	68.2 (10.0)	23.76	35–40	Running, Cycling, Swimming, or Aerobic dancing
(Franzoni et al., 2005)	Young Control	16	M	34.1 (7.5)	–	–	23.1	–	–
	Older Control	16	M	63.7 (4.3)	–	–	24.2	–	–
	Young Endurance	16	M	33.4 (6.7)	–	–	23.4	11 (2)	Endurance Running
	Master Endurance Athlete	16	M	63.6 (6.1)	–	–	23.9	37 (5)	Endurance Running
(Galetta et al., 2005)	Master Endurance Athlete	20	M	68.5 (4.5)	–	–	23.4	> 40	Endurance Running
	Older Control	20	M	68.2 (3.7)	–	–	24.1	–	–

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Author, Year	Participant Groups	Number (n)	Sex (M/F)	Age (Years)	Height (m)	Weight (kg)	BMI (Kg.m^2)	Training Experience (years)	Type of Training
(Galetta et al., 2006)	Older Control	28	M	65.6 (5.6)	5.60	–	–	–	–
	Master Endurance Athlete	30	M	64.5 (4.5)	4.50	–	–	> 40	Endurance Running
(Hawkins et al., 2001)	Master Endurance Athlete	34	M	62.2 (3.5)	1.77 (0.02)	77.0 (7.6)	24.5	22.6 (9.3)	Endurance Running
	Master Endurance Athlete	13	M	71.1 (3.2)	1.73 (0.03)	67.5 (9.4)	22.7	21.6 (11.5)	Endurance Running
	Master Endurance Athlete	8	M	82.8 (4.0)	1.73 (0.02)	68.9 (5.9)	23.0	25.9 (22.3)	Endurance Running
(Hayes et al., 2015)	Older Control	28	M	63.0 (5.0)	1.75 (0.06)	90.4 (18.1)	29.5	–	–
	Master Endurance Athlete	20	M	60.0 (5.0)	1.74 (0.06)	79.3 (13.3)	26.2	> 30	Water-polo/Triathlon/Sprint cycling/Road cycling/Distance running
(Katzel et al., 1998)	Master Endurance Athlete	70	M	63.0 (6.0)	–	70.0 (8.0)	23.0	> 20	Running/Triathlon/Cycling/Tennis
	Older Control	85	M	61.0 (7.0)	–	83.0 (11.0)	27.0	–	
(Katzel et al., 2001)	Master Endurance Athlete	42	M	63.4 (6.5)		69.4 (7.8)	23.1	> 20	Running/Triathlon/Cycling/Tennis
	Older Control	47	M	61.1 (6.2)		91.4 (11.7)	29.3	–	–
(Korhonen et al., 2006)	Young Power	16	M	24.3 (4.0)	1.78 (0.04)	77.2 (5.6)	24.4	13.2 (5.2)	Sprint/Strength
	Master power athletes	21	M	65.8 (2.8)	1.73 (0.04)	77.2 (4.1)	25.9	35.1 (19.3)	Sprint/Strength
	Master power athletes	20	M	75.3 (4.0)	1.71 (0.05)	69.8 (8.9)	23.8	34.3 (21.9)	Sprint/Strength
(Korhonen et al., 2012)	Young Control	19	M	36.2 (4.4)	1.82 (0.06)	79.2 (6.8)	24.0	–	Ball games/Jogging/Cross-country skiing
	Master power athletes	24	M	65.9 (2.6)	1.72 (0.04)	71.1 (5.6)	24.0	35.1 (18.4)	Sprint/Strength
	Master power athletes	24	M	75.8 (4.3)	1.71 (0.06)	70.3 (8.5)	24.1	34.3 (21.4)	Sprint/Strength
(Larsson et al., 1997)	Young control	4	M	25–31	–	–	–	–	–
	Older Control	2	M	73–81	–	–	–	–	–
	Master Endurance Athlete	2	M	73–81	–	–	–	> 50	Endurance Trained/Competitive Wrestler
(Mackey et al., 2014)	Young Control	12	M	24.0 (3.0)	1.78 (0.06)	70.0 (8.0)	22.2	–	–
	Young Endurance	10	M	26.0 (4.0)	1.79 (0.04)	73.0 (6.0)	22.7	6.0 (2.0)	Endurance Running
	Older Control	12	M	66.0 (4.0)	1.75 (0.04)	75.0 (4.0)	24.5	–	–
	Master Endurance Athlete	15	M	64.0 (4.0)	1.76 (0.05)	71.0 (6.0)	22.9	28.0 (9.0)	Endurance Running
(Marcell et al., 2003)	Master Endurance Athlete	9	M	67.1 (1.2)		70.2 (4.8)	23.2	23.6 (12.6)	Endurance Running
(Marcell et al., 2014)	Master Endurance Athlete	21	M	71.3	1.74	71.3	23.5	24.9	Endurance Running
(Matelot et al., 2016)	Master Endurance Athlete	13	M	62.0 (3.0)	1.72 (0.04)	71.2 (6.1)	24.1	39.0 (4.0)	Endurance Running/Cycling
(Mikkelsen et al., 2013)	Young Control	12	M	24.0 (3.0)	1.78 (0.06)	70.0 (8.0)	22.0	–	–
	Young Endurance	10	M	26.0 (4.0)	1.79 (0.04)	73.0 (6.0)	23.0	6.0 (3.16)	Endurance Running
	Older Control	12	M	66.0 (4.0)	1.75 (0.04)	75.0 (4.0)	25.0	–	–
	Master Endurance Athlete	15	M	64.0 (4.0)	1.76 (0.04)	71.0 (6.0)	23.0	28.0 (7.75)	Endurance Running
(Molmen et al., 2012)	Older Control	10	M	71.7 (1.3)		76.5 (9.4)	25.0		
	Young Control	10	M	24.8 (2.3)		76.0 (16.0)	22.7		
	Master Endurance Athlete	11	M	74.3 (1.8)		74.5 (8.3)	23.0	> 25	Cross Country Skiers
(Mortensen et al., 2012)	Young Control	8	M	23.0 (2.8)	1.83 (0.01)	79.0 (11.3)	23.6	–	–
	Older Control	8	M	66.0 (5.7)	1.75 (0.01)	79.0 (5.7)	25.8	–	–
	Master Endurance Athlete	8	M	62.0 (5.7)	1.78 (0.01)	76.0 (8.5)	24.0	> 30	Endurance trained

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Author, Year	Participant Groups	Number (n)	Sex (M/F)	Age (Years)	Height (m)	Weight (kg)	BMI (Kg.m^2)	Training Experience (years)	Type of Training
(Mosole et al., 2014)	Young Power	5	M	26.2 (4.0)	–	–	–	> 5.0	Weightlifting
	Older Control	6	M = 4 F = 2	71.8 (3.5)	–	–	–	–	–
	Master Endurance Athlete	7	M	68.3 (4.0)	–	–	–	> 20	Endurance/Mixed
(Mucci et al., 1999)	Young Athletes	7	M	26.1 (3.4)	1.78 (0.10)	67.8 (7.7)	21.4	4.0 (2.38)	Triathlon/Endurance Running
	Young Control	7	M	23.0 (4.0)	1.83 (0.09)	73.3 (10.1)	21.9	–	–
	Master Endurance Athlete	7	M	64.4 (10.9)	1.69 (0.13)	72.3 (11.1)	25.4	25 (5.29)	Cycling
	Older Control	7	M	61.6 (3.4)	1.69 (0.04)	69.6 (4.2)	24.4	–	–
(Nyberg et al., 2012)	Young Control	8	M	23.0 (2.8)	1.83 (0.06)	79.4 (12.2)	23.7	–	–
	Older Control	8	M	66.0 (5.7)	1.75 (0.08)	79.2 (5.1)	25.9	–	–
	Master Endurance Athlete	8	M	62.0 (5.7)	1.78 (0.06)	75.7 (8.8)	23.9	> 30	Endurance trained
(Ojanen et al., 2007)	Master power athletes	12	M	60.8 (2.1)	1.78 (0.08)	94.2 (13.4)	29.8	27.5 (14.8)	Shotput/Discus/Hammer
	Master power athletes	9	M	75.0 (4.9)	1.77 (0.05)	87.4 (11.0)	27.8	27.2 (19.8)	Shotput/Discus/Hammer
	Older Control	10	M	61.1 (2.7)	1.77 (0.06)	80.0 (12.2)	25.5	–	–
	Older Control	5	M	69.2 (3.7)	1.75 (0.06)	75.7 (11.1)	24.7	–	–
(Pollock et al., 2015)	Master Endurance Athlete	24	M	62.0 (1.4)	1.70 (0.06)	76.8 (8.5)	24.7	27.6 (17.5)	Cycling
	Master Endurance Athlete	19	M	67.0 (1.2)	1.77 (0.06)	72.6 (7.2)	23.2	26.5 (19.6)	Cycling
	Master Endurance Athlete	19	M	73.4 (2.6)	1.76 (0.06)	74.4 (10.1)	24.1	36.7 (19.5)	Cycling
	Master Endurance Athlete	15	F	61.9 (1.6)	1.66 (0.06)	60.4 (5.5)	22.1	24.6 (20.3)	Cycling
	Master Endurance Athlete	4	F	75.3 (3.0)	1.61 (0.07)	57.1 (6.4)	22.0	45 (19.6)	Cycling
(Power et al., 2012)	Young Control	9	M	27.0 (5.0)	1.81 (0.07)	80.8 (9.6)	24.6	–	–
	Older Control	9	M	70.0 (5.0)	1.78 (0.07)	90.1 (17.4)	28.5	–	–
	Master Endurance Athlete	9	M = 8 F = 1	67.0 (4.0)	1.74 (0.06)	71.3 (10.5)	23.5	> 30	Endurance Running
(Power et al., 2010a)	Young Control	10	M	27.0 (3.0)	1.78 (0.08)	80.7 (10.0)	25.6	–	–
	Older Control	10	M	66.0 (3.0)	1.72 (0.07)	78.8 (10.4)	26.7	–	–
	Master Endurance Athlete	10	M = 9 F = 1	64.0 (3.0)	1.77 (0.07)	72.3 (7.7)	23.0	38.2 (6.7)	Endurance Running
(Prasad et al., 2007)	Young Control	12	M = 9 F = 3	32.3 (9.0)	–	–	–	–	–
	Older Control	13	M = 7 F = 6	69.8 (3.0)	–	–	–	–	–
	Master Endurance Athlete	12	M = 6 F = 6	67.8 (3.0)	–	–	–	23.0 (8.0)	Marathons, Triathlons, Middle distance
(Prefaut et al., 1994)	Master Endurance Athlete	10	M	65.3 (8.2)	1.68 (0.07)	70.1 (4.3)	24.8	33.0 (18.0)	Cycling
	Older Control	10	M	68.3 (7.0)	1.72 (0.05)	74.8 (7.6)	25.3	–	–
	Young Endurance	10	M	23.3 (3.5)	1.80 (0.03)	68.8 (6.0)	21.2	–	–
(Proctor and Joyner, 1997)	Young Endurance	8	M	24.0 (4.0)	1.80 (0.07)	70.9 (7.8)	21.9	9.0 (3.0)	Running, Cycling, Triathlon Running, Cycling, Triathlon Running, Cycling, Triathlon
	Master Endurance Athlete	8	M	64.0 (4.0)	1.78 (0.06)	75.5 (10.2)	23.8	21.0 (5.0)	
	Young Endurance	8	F	26.0 (4.0)	1.71 (0.05)	60.1 (5.5)	20.6	9.0 (5.0)	
(Rantalainen et al., 2014)	Young Power	26	M	27.4 (5.1)	1.81 (0.05)	76.7 (5.9)	23.4	15.4 (6.2)	Sprinting
	Young Control	41	M	28.7 (5.8)	1.8 (0.06)	78.1 (9.6)	24.1	–	–
	Master power athletes	35	M	72.4 (5.3)	1.71 (0.05)	70.7 (7.1)	24.2	35.4 (19.1)	Sprinting
	Older Control	24	M	71.6 (4.2)	1.71 (0.06)	75.8 (8.6)	25.9	–	–

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Author, Year	Participant Groups	Number (n)	Sex (M/F)	Age (Years)	Height (m)	Weight (kg)	BMI (Kg.m^2)	Training Experience (years)	Type of Training
(Rivier et al., 1994)	Young Endurance	10	M	23.9 (3.6)	1.78 (0.05)	69.4 (5.8)	21.8	–	Triathlon
	Master Endurance Athlete	6	M	63.7 (5.0)	1.67 (0.06)	75.3 (4.0)	27.0	30.0 (5.0)	Cycling
(Sallinen et al., 2008)	Young Control	10	M	25.7 (3.4)	1.82 (0.04)	77.0 (5.2)	23.3	–	–
	Master power athletes	8	M	71.8 (3.8)	1.75 (0.06)	86.3 (10.7)	28.4	22.8 (14.9)	National level throwers
	Older Control	10	M	70.6 (3.3)	1.70 (0.06)	71.4 (71.4)	24.7	–	–
(Sanada et al., 2009)	Young Control	23	M	25.3 (2.7)	–	70.8 (11.2)	23.5	–	–
	Older Control	22	M	65.2 (4.1)	–	69.7 (8.1)	24.3	–	–
	Young Endurance	26	M	20.3 (1.0)	–	69.3 (5.7)	22.3	3.0	Rowing
	Master Endurance Athlete	24	M	65.7 (3.0)	–	67.9 (8.2)	23.0	46.7 (2.8)	Rowing
(Schmidt et al., 2015)	Master Endurance Athlete	17	M	68.1 (2.1)	1.78 (0.03)	78.1 (8.2)	24.6	52.0 (11.0)	Football
	Older Control	26	M	68.2 (3.2)	1.76 (0.03)	84.1 (11.1)	27.2	–	–
(Shibata and Levine, 2012)	Older Control	10	M = 6 F = 4	71.0 (3.0)	1.71 (0.09)	74.0 (10.0)	26	–	–
	Master Endurance Athlete	11	M = 5 F = 6	68.0 (3.0)	1.70 (0.12)	65.0 (14.0)	22.1	23.0 (8.0)	Endurance Running
(Sipila and Suominen, 1991)	Master power athletes	7	M	77.1 (3.5)	1.69 (0.06)	69.8 (9.3)	–	30 to 70	Track and field, Gymnastics
	Master Endurance Athlete	14	M	74.2 (3.0)	1.71 (0.07)	68.7 (8.6)	–	30 to 70	Running, Cross-country Skiing, Cycling, Swimming
	Older Control	11	M	73.4 (2.4)	1.68 (0.04)	74.7 (11.6)	–	–	–
(Stenroth et al., 2016)	Young Control	18	M	23.7 (2.0)	1.81 (0.06)	75.4 (9.0)	23.1	–	–
	Older Control	33	M	74.8 (3.6)	1.73 (0.05)	76.1 (7.7)	25.4	–	–
	Master Endurance Athlete	10	M	74.0 (2.8)	1.75 (0.07)	69.9 (6.9)	22.7	39.4 (20.9)	Endurance Exercise
	Older Power	10	M	74.4 (2.8)	1.76 (0.07)	74.3 (7.1)	24.1	44.7 (19.7)	Sprinting
(Sundstrup et al., 2010)	Master Endurance Athlete	10	M	69.6 (4.4)	1.77 (0.05)	83.7 (8.9)	26.8	50.0	Football
	Young Control	49	M	32.4 (6.3)	1.82 (0.05)	87.7 (12.6)	26.5	–	–
	Older Control	8	M	70.5 (2.8)	1.75 (0.06)	82.9 (6.2)	27.1	–	–
(Suominen and Rahkila, 1991)	Master Endurance Athlete	67	M	73.7 (2.7)	1.71 (0.07)	69.3 (8.6)	23.8	–	Endurance Running, Orienteers, Cross-country Skiers
	Master power athletes	14	M	74.3 (2.9)	1.74 (0.06)	82.4 (12.5)	27.1	–	Throwers, Weight Lifters
	Master power athletes	16	M	75.5 (3.8)	1.70 (0.06)	69.4 (12.0)	24.1	–	Sprinters, Jumpers
	Older Control	42	M	74.2 (2.8)	1.69 (0.07)	76.3 (12.0)	26.7	–	
(Tarpenning et al., 2004b)	Master Endurance Athlete	29	M	64.2 (2.3)	1.76 (0.07)	71.5 (8.1)	23.1	20.33 (9.0)	Endurance Running
	Master Endurance Athlete	11	M	74.6 (3.5)	1.74 (0.06)	68.6 (7.0)	22.6	24 (20.6)	Endurance Running
(Thomas et al., 2013)	Master Endurance Athlete	10	M = 7 F = 3	74.5 (5.8)	–	–	–	23.0 (8.0)	Endurance Running, Swimming, Cycling
	Older Control	10	M = 8 F = 2	75.4 (5.6)	–	–	–	–	–
	Young Control	9	M = 5 F = 4	27.0 (3.6)	–	–	–	–	–
(Trappe et al., 2013)	Master Endurance Athlete	9	M	81.0 (3.0)	1.72 (0.06)	68.0 (9.0)	23.0	> 50	Cross-country Skiing, Orienteering, Track and Field
	Older Control	6	M	82.0 (4.9)	1.72 (0.10)	77.0 (12.2)	26.0	–	–
(Witkowski et al., 2010)	Older Control	11	M	64.0 (6.6)	1.76 (0.07)	73.0 (8.0)	23.6	–	–
	Master Endurance Athlete	12	M	62.0 (6.9)	1.78 (0.10)	70.1 (10.0)	22.0	32.0 (10.4)	Endurance Training
(Yataco et al., 1997)	Master Endurance Athlete	61	M	63.3 (6.1)	–	70.1 (7.1)	22.9	> 20	Running/Cycling/Swimming
	Older Control	39	M	60.6 (5.6)	–	77.1 (8.5)	25.6		

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Author, Year	Participant Groups	Number (n)	Sex (M/F)	Age (Years)	Height (m)	Weight (kg)	BMI (Kg.m^2)	Training Experience (years)	Type of Training
(Zampieri et al., 2015)	Young Control	5	M	27.3 (4.2)	1.75 (0.04)	73.8 (5.9)	24.2	–	–
	Older Control	9	M	71.4 (3.0)	1.77 (0.08)	84.9 (10.1)	26.9	–	–
	Master Endurance Athlete	15	M	70.2 (4.0)	1.76 (0.05)	81.7 (8.8)	26.3	> 30	Endurance, Strength, Power and Games

Table 2. Anthropometric characteristics and training background of study groups (mean ± SD) included in this review, (e.g. n, age, height, weight, BMI, years of training, and type of training undertaken).

Study Group	Fat-Free Mass (kg)	Fat Mass (kg)	Body Fat (%)	Muscle Thickness (cm)	Fascicle Length (cm)	Muscle Cross-Sectional Area (mm^2)	Muscle Fibre Area (μm^2)	Muscle Fibre Distribution (%)
Young Controls	53.8 ± 5.7 (1, n = 23)	–	17.4 ± 3.0 (8, n = 126)	–	–	MRI: 6792 ± 696 (n = 12)	Type I: 4282 ± 26 (2, n = 28), Type 2: 4783 ± 872 (1, n = 12) Type IIa: 4700 ± 560.0 (1, n = 16) Type IIx: 3200 ± 2720 (1, n = 16) Type IIax: 3700 ± 760 (1, n = 16)	Type I: 43.5 ± 4.5 (4, n = 37) Type 2: 53.5 ± 5.0 (2, n = 17) Type IIa: 35 ± 12 (1, n = 16) Type IIx: 7.5 ± 6.4 (2, n = 20) Type IIax: 12.0 ± 00 (2, n = 20) Denervated: (0.3 ± 0.0 (1, n = 5)
Young Endurance	54.8 ± 7.3 (4, n = 56)	12.0 ± 4.2 (1, n = 14)	14.8 ± 4.9 (8, n = 89)	–	–	MRI: 7859 ± 636 (n = 10)	Type I: 5550 ± 1337 (1, n = 10) Type 2: 5498 ± 1559 (1, n = 10)	Type I: 51.5 ± 13.4 (2, n = 15) Type 2: 48.6 ± 13.5 (2, n = 15) Type IIax: 0.5 ± 0.6 (1, n = 5) Denervated: 0.4 ± 0.5 (1, n = 5)
Young Power	–	–	–	VL: 2.61 ± 0.08 (n = 16)	VL: 7.91 ± 0.49 (n = 16)	–	–	–
Older Controls	54.5 ± 7.7 (5, n = 122)	20.1 ± 6.3 (2, n = 65)	24.5 ± 4.6 (20, n = 368)	RF: 2.8 ± 0.56 (n = 11)	–	MRI: 5504 ± 727 (n = 12) US: 4840 ± 1110 (n = 11)	Type I: 5488 ± 459 (3, n = 28) Type 2: 4149 ± 903 (1, n = 12) Type IIa: 5411 ± 484 (2, n = 16) Type IIx: 3974 ± 0 (2, n = 16) Type IIax: 4570 ± 980 (1, n = 4)	Type I: 49.8 ± 4.2 (6, n = 45) Type 2: 50.3 ± 4.0 (3, n = 27) Type IIa: 23.5 ± 0 (2, n = 16) Type IIx: 22.2 ± 13.1 (3, n = 18) Type IIax: 5.9 ± 5.8 (2, n = 8) Denervated: 5.3 ± 1.8 (2, n = 15)
Master Endurance	55.2 ± 6.1 (19, n = 366)	14.1 ± 3.6 (3, n = 91)	19.2 ± 4.1 (38, n = 595)	RF: 2.77 ± 0.39 (n = 14)	–	MRI: 6481 ± 775 (n = 15) US: 5270 ± 880 (n = 14)	Type I: 5367 ± 588 (5, n = 74), Type 2: 4478 ± 347 (3, n = 55), Type IIa: 5043 ± 41 (2, n = 19), Type IIx: 3955 ± 88 (2, n = 19), Type IIax: 3590 ± 687 (1, n = 7)	Type I: 60.8 ± 10.7 (7, n = 96), Type 2: 38.4 ± 11.3 (5, n = 77), Type IIa: 32.6 ± 10.7 (2, n = 19), Type IIx: 10.3 ± 2.7 (3, n = 21), Type IIax: 6.3 ± 8.1 (2, n = 9), Denervated: 1.9 ± 0.1 (2, n = 22)
Master Power	–	–	19.6 ± 5.8 (8, n = 125)	VL 60–69: 2.1 ± 0.09 (n = 21), VL 70–84: 1.96 ± 0.08 (n = 20), RF: 2.73 ± 0.37(n = 7)	VL 60–69: 7.99 ± 0.27 (n = 21), VL 70–84: 7.38 ± 0.27 (n = 20)	US: 5250 ± 1080 (n = 7)	Type I: 4750 ± 1273 (4, n = 34), Type IIa: 4755 ± 1404 (4, n = 34), Type IIx: 4019 ± 990 (4, n = 34), Type IIax: 4120 ± 495 (3, n = 27)	Type I: 48.0 ± 8.0 (5, n = 59), Type IIa: 31.8 ± 38 (3, n = 59), Type IIx: 10.7 ± 4.1 (5, n = 59), Type IIax: 11.6 ± 2.6 (2, n = 52)

Table 3. Body composition, muscle mass and morphology (mean ± SD) not included in a meta-analysis (e.g. fat-free mass, fat-mass, relative fat-mass (additional data ineligible to be included in meta-analysis), muscle thickness, fascicle length, muscle CSA, fibre area, fibre distribution). First number in the bracket indicates the number of studies that assessed that particular variable, the second number indicates the number of individuals assessed.

3. Physiological Function and Skeletal Muscle Morphology in Chronically Trained Master Endurance Athletes Compared with Untrained Older and Younger Individuals

3.1. ABSTRACT

Background: To better understand the role of inherent ageing and biological factors in the progression of age-related muscle deterioration, and the potency of exercise training for enhancing physiological function and health, we conducted a comprehensive phenotypic comparison of chronically exercise trained older individuals (Master Athletes; MA) against untrained young and older individuals.

Methods: In a cross-sectional design 15 young controls (YC) (20±2.7 y, 78.9±13.3 kg), 12 untrained older controls (OC) (69.8±4.1 y, 77.5±14.2 kg) and 14 endurance trained MA (67.1±4.1 y, 68.7±6.5 kg) underwent assessments of body composition, maximal aerobic fitness, leg strength, muscle architecture and fibre-type morphology.

Results: Skeletal muscle index was lower and body fat greater in OC vs. MA ($P=0.001$ and $P<0.002$, respectively). VO_{2max} was similar between MA and YC, but lower in OC ($P<0.001$). Maximal isometric leg strength was greater in YC than OC and MA ($P<0.001$ and $P=0.006$, respectively). Type I fibre area was greater for MA compared with OC ($P=0.028$), whilst Type II fibre area was greater for YC compared with OC ($P=0.008$). Type II fibre myonuclear domain size was greater in YC than OC and MA ($P=0.005$ and $P=0.016$, respectively), whereas satellite cell content was similar between groups. MA had greater Type I and Type II fibre capillarisation compared with OC and YC.

Conclusions: Body composition, physiological function and muscle fibre morphology generally deteriorated in OC compared with YC, whereas MA were generally indistinguishable from YC. Thus, chronic exercise training enhances skeletal muscle morphology and supports superior physiological health in older age.

3.2. INTRODUCTION

The shift towards an ageing population presents a significant and overwhelming global demand on healthcare resources. The major trepidation is not that individuals are living longer (i.e. lifespan), but that they endure a larger portion of their later years with multiple age-associated co-morbidities (i.e. healthspan) (Harper, 2014). Thus, strategies to close the gap between health- and life-span are of paramount importance.

Age-related reductions of skeletal muscle mass, strength and function (termed 'sarcopenia') and cardiorespiratory fitness may prematurely force individuals into a state of physical dependence and are independent predictors of all-cause mortality (Blair et al., 1996, Kelley and Kelley, 2017, Newman et al., 2006). Skeletal muscle mass is indispensable for locomotion, basal metabolism, energy storage and nutrient deposition (Frontera and Ochala, 2015). By the 8th decade of life skeletal muscle mass has is reduced by ~18% in men and 27% in women (Janssen et al., 2000) and is accompanied by a loss of muscle strength that is ~3-5 times greater (Goodpaster et al., 2006). Sarcopenia is characterised by reductions in muscle fibre cross-sectional area (CSA) (Nilwik et al., 2013), satellite cell content (Nederveen et al., 2016), motor unit remodelling (Piasecki et al., 2018), infiltrations of fat and connective tissue (Smeuninx et al., 2017), alterations to the microcirculation (Barnouin et al., 2017, Pollock et al., 2018, Nederveen et al., 2016) and reduced oxidative capacity (Wilson and Tanaka, 2000). The extent to which this physiological deterioration is due to inherent biological processes (primary ageing), free from artefacts of secondary ageing that exacerbate the decline (i.e., physical inactivity), is unclear.

From an evolutionary biological perspective, the default state of human well-being is a lifestyle incorporating physical activity/exercise (Booth and Lees, 2006). Therefore,

the study of individuals who have chronically undertaken structured exercise training and continue to compete, into their later years, referred to as Master Athletes (MA), provides a model to investigate the impact of inherent ageing on physiological function, without confounding aspects of biological ageing. In a recent meta-analysis, we reported that endurance-based MA displayed a 55% greater aerobic capacity compared with age-matched untrained individuals, whereas resistance trained MA demonstrated greater strength than age-matched untrained individuals (McKendry et al., 2018). Furthermore, others have shown that although a decline in physiological function remains apparent in MA, high physical activity may ultimately improve the healthspan by shifting the 'set point' of age-related physiological deterioration upwards and to the right (Harridge and Lazarus, 2017, Pollock et al., 2015, Pollock et al., 2018). Nevertheless, firm conclusions on the influence of ageing and chronic exercise on physiological deterioration cannot be drawn, as very few studies have included sufficient parameters to comprehensively characterise skeletal muscle morphology at macro- and microscopic level in MA (Mackey et al., 2014, Zampieri et al., 2015, Piasecki et al., 2016a). Studies that have characterised MA report conflicting findings, with some demonstrating superior leg strength and muscle fibre diameter in senior sportsmen compared with untrained older individuals (Zampieri et al., 2015), and others showing similar muscle size, strength and fibre CSA between MA and untrained older individuals (Mackey et al., 2014, Piasecki et al., 2016a). These discrepant findings may relate to different analytical methods, the specific muscle studied or the population selected for comparison against MA.

Therefore, to better understand the role of inherent ageing processes in physiological function, and the extent to which chronic exercise training might improve the healthspan, the aim of the current study was to compare physical function,

cardiorespiratory fitness, body composition, muscle strength, architecture and fibre-type morphology in MA, with healthy untrained younger and age-matched older individuals. We hypothesised that untrained older individuals would exhibit an impairment in all of the above parameters compared with younger individuals, which would be partially or, in some cases, completely absent in MA.

3.3. METHODS

3.3.1. Participants

Fifteen young (YC) and 12 older untrained controls (OC) were recruited alongside 14 Master endurance athletes (MA) through local advertisements, the British Masters Athletics Federation and the League of Veteran Racing Cyclists. Young (18-35 years) and older untrained controls (60-80 years) were deemed eligible for study participation only if they maintained habitual activity and had not previously participated in any form of structured exercise training outside of recreational activities. Master athletes (60-80 years) were included only if they had maintained continuous endurance training at least twice per week for ≥20 years preceding the study. Participant anthropometric and training characteristics are detailed in Table 1. All participants were informed of the purpose and methodology of the study, were deemed healthy by completion of a general health questionnaire assessment, and provided their written informed consent. Ethical approval was obtained through the West Midlands - Solihull Research Ethics Committee (16/WM/0167). The study conformed to the standards set by the Declaration of Helsinki (7^{th} version).

3.3.2. Study design

In a parallel study design, three divergent groups YC, OC and MA were recruited to investigate the effect that continuous endurance exercise exerts on indices of muscle mass, function and morphology. Following initial study screening and consenting, participants reported to the School of Sport, Exercise and Rehabilitation Sciences (SportExR) laboratory on two separate occasions with each visit separated by ~7 days. For each visit participants reported to SportExR in an overnight fasted-state, having refrained from strenuous physical activity and alcohol for at least 48 h previously, and from caffeine consumption on the day of the trial. During the initial visit, participants underwent assessments of body composition, aerobic capacity, maximal limb strength and a battery of functional tests. Approximately 1 week later, participants underwent ultrasound scanning, a single venous blood sample and a single muscle biopsy.

3.3.2.1. Visit 1

Body mass, height and composition: Body mass was determined by weighing each participant in loose clothing, without shoes, to the nearest 0.1kg using a digital balance scale (Esca 813, Hamburg, Germany). Height measurements were made to the nearest 0.1cm using a stadiometer (Seca 217, Hamburg, Germany). Participants underwent a dual-energy x-ray absorptiometry (DXA) scan (Discovery DXA Systems, Hologic Inc., Bedford, MA) to determine whole-body and regional fat- and fat-free mass.

Blood pressure: Blood pressure was measured using a standard fully automatic blood pressure monitor (OMRON M2, OMRON Healthcare UK Ltd., UK). Participants were asked to remove any clothing that obstructed the blood pressure cuff. Participants were

seated with their legs uncrossed and back supported, encouraged to relax and refrain from talking during the assessment. This test was repeated 3 times and the highest reading taken.

Aerobic capacity: Participants underwent the Ekblom-Bak test (Bjorkman et al., 2016); a submaximal cycle ergometry test for the estimation of $\dot{V}O_{2max}$. Briefly, the test is based on the change in heart rate between a standardised low workload and a higher workload predetermined by sex and current habitual activity. Participants were instructed to maintain a constant cadence of 60 rpm throughout the test. First, the participant cycled for 4 min, while investigators ensured constant cadence and resistance. Heart rate was recorded every 15 s and averaged during the final minute of the low workload. The Electrocardiogram (ECG) was monitored over 5 min prior-to and continuously throughout exercise by a cardiologist. The resistance was then increased to the predetermined level for the next 4 min, whilst cadence and resistance remained constant. During the second minute at the higher workload, individuals communicated their rate perceived exertion (RPE) using a standard scale (Borg, 1982). If RPE was <10 the resistance was increased by 1kp and the second workload timings started again. If RPE was 10-11, the workload was increased by 0.5kp and the second workload timings started again. If RPE was 12-16 participants were instructed to maintain this workload. If RPE >17 the exercise bout was stopped. Heart rate was recorded every 15 s and averaged during the final minute of the higher workload. $\dot{V}O_{2max}$ was calculated using the following equation (Bjorkman et al., 2016):

$$\dot{V}O_{2max} = 4.98196 - 2.88618\left(\frac{\Delta HR}{\Delta PO}\right) + 0.65015(Sex) - 0.01712(Age)$$

Where ΔHR is the difference in the average heart rate between the two workloads, ΔPO is a constant reflecting the difference in power output between the two workloads, where sex is 0=woman and 1=man, and age is participants age in years as an integer.

Handgrip strength: Individuals stood, feet side-by-side, with their arms adducted, wrists neutrally rotated and the dynamometer (Jamar Hydraulic, Patterson Medical, UK) positioned comfortably in the participant's self-reported dominant hand. Participants were instructed to squeeze the handle as hard as possible and three attempts were recorded, with the highest value included in future analysis.

Short physical performance battery (SPPB): This battery of tests assesses three areas critical to successful and independent function with advancing age; balance, gait speed and low limb power (Guralnik et al., 1994). The tests were always performed in the same order. First, the balance test was comprised of three separate, increasingly difficult stances (side-by-side, semi-tandem, full tandem), which participants were required to hold for 10 sec. Second, the gait speed test in which participants were instructed to walk a short, 3-metre-long course 'at your usual speed as if walking to the shops'. Finally, a repeated chair stand test, in which participants were instructed to rise from a chair, arms folded, five times as quickly as possible. The result from each of the three tests were scored out of 4 marks (maximum total of 12), against predetermined criteria to provide an overall score of physical function.

Limb strength: Maximal knee extension and elbow flexion isometric strength were measured using a KinCom Dynamometer (KinCom 125AP, KinCom, USA) on the self-

reported dominant limb. Participants were seated, with the tested limb, chest and hips stabilised and with the knee and elbow angle positioned at 70 degrees of flexion, respectively. Participants were allowed 3 practices of increasing exertion to familiarise themselves with the isometric contraction. After this, participants performed 3 maximal voluntary contractions, the greatest of which was included in subsequent analysis.

Dietary analysis: Between visits 1 and 2, participants were provided with a 4-day weighed food diary designed to capture habitual food intake on 2 consecutive weekdays and 2 weekend days. Participants were instructed not to change their usual diet and to be as accurate as possible when describing the food (cooking method, brand, amount etc.). Dietary intake was analysed using MyFitnessPal software (MyFitnessPal Inc.).

Physical Activity: Between visits 1 and 2 (~7 days), participants were provided with a wrist-worn accelerometer (GENEActiv, ActivInsights Ltd., UK) designed to capture habitual activity in free-living conditions over 5 consecutive days (including both weekend days). Accelerometers were initialized to sample data at a 10 Hz. Data were converted into 60 second epochs and analysed using the GENEActiv software (version 2.2, ActivInsights). Activities were split into 4 categories based on metabolic equivalent (MET) values; i) sedentary activity (<1.5 METs) ii) light activity (1.5 – 3.99 METs) iii) moderate activity (4.0 – 6.99 METs) and iv) vigorous activity (>7 METs) (Esliger et al., 2011).

3.3.2.2. Visit 2

Muscle architecture: B-mode ultrasonography with a linear array probe was used for all measurements of muscle architecture. Participants lay fully relaxed in a supine position with a small towel rolled and placed under the knee for anterior measurements. For posterior measurements, participants were in a fully relaxed prone position with feet overhanging the end of the bed. Biceps Brachii architecture was measured with the participants seated on the edge of the bed with the arm freely hanging. The muscles measured included; *Vastus Lateralis, Vastus Intermedius, Rectus Femoris, Biceps Brachii, Gastrocnemius Medialis, and Tibialis Anterior.* Measurements of muscle thickness (MT), pennation angle (θ), fascicle length (L_f) were made in triplicate. MT was considered to be the distance between deep and superficial aponeuroses. θ was calculated as the angle between the muscle fascicle and the deep aponeurosis. Fascicle length was measured as the length of the fascicular path between the 2 aponeuroses. A mark was drawn on each pre-determined anatomical site to ensure constant probe positioning. Specifically, the measurement sites were set at 60% of the upper arm length distal to the acromion process for Biceps Brachii, 50% of the muscle length for upper thigh muscles, and 30% of the leg length distal to the popliteal crease for lower leg muscles (Kawakami et al., 2006). The ultrasonography probe was covered with water-soluble transmission gel, to provide acoustic contact without compressing the dermal surface, and an image was taken when a number of fascicles could clearly be identified. The orientation of the probe varied slightly between participants due to the individual characteristics.

Blood sample: A single blood sample was obtained via venepuncture from an antecubital forearm vein. Blood was collected in separate ethylenediaminetetraacetic acid (EDTA)

and serum-separating polymer gel containing BD vacutainers (BD, Oxford, UK). Blood samples were centrifuged at 3000g for 10 min at 4°C, and serum and plasma aliquots were frozen at −80°C for later analysis.

Muscle Biopsy: A muscle biopsy sample was obtained from the quadriceps *vastus lateralis* under local anaesthesia (1% lidocaine) using the Bergström needle technique (Bergstrom, 1975). Muscle biopsy tissue was quickly rinsed in ice-cold saline and blotted to remove any visible fat and connective tissue before being frozen in liquid nitrogen or placed in a pipette tip with optimum cutting temperature compound (Tissue-Tek® (O.C.T.) Compound, Sakura® Finetek) and frozen in liquid nitrogen-cooled isopentane and stored at −80°C for later analysis.

3.3.3. Analysis

Blood: Plasma glucose was analysed using a commercially available blood glucose analyser (HemoCue® Hb 201+ System, HemoCue AB, Sweden). Serum insulin (Coefficient of variation (CV) = 2.65 ± 1.46%) and C-reactive protein (CRP) (CV = 4.68 ± 1.10%) were analysed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (IBL International GmbH, Hamburg, Germany) following the manufacturer instructions. Postabsorptive insulin sensitivity was also estimated using the homeostasis model of insulin resistance index (HOMA-IR) (Matthews et al., 1985).

Immunohistochemistry:

i) Muscle Fibre Type and Cross-sectional area: This analysis was carried out on a subset of participants (YC; *n*=13: MA; *n*=14: OC; *n*=11). Serial 10µm sections of skeletal muscle biopsies were cut in the cryostat at -20°C. Briefly, slides were washed in triton x100 (0.02 %) to permeabilise the fibres. Slides were blocked for 90 mins in 5 % normal goat serum (Invitrogen, UK) and then incubated overnight in the primary antibody cocktail; myosin heavy chain (MHC) I, BAF8 (Developmental Studies Hybridoma Bank (DSHB, USA) 1:25; MHC II, SC-71 (DSHB, USA) 1:20; Laminin (ab11575) 1:500 (Abcam, Cambridge, UK) in phosphate buffered saline (PBS). Slides were washed in 3 x 5 min in PBST and incubated in the secondary antibody cocktail; MHC I, Alexa Fluor 546 IgG2b 1:100 (Thermo Fisher Scientific, Paisley, UK); MHC II, Alexa fluor 488 IgG1 1:100 (Thermo Fisher Scientific, Paisley, UK); Laminin, Alexa Fluor 488 IgG 1:100 (Thermo Fisher Scientific, Paisley, UK). Slides were then washed and mounted with coverslips in prolong gold anti-fade mountant (Thermo Fisher Scientific, Paisley, UK). Prepared slides were observed under a Nikon E600 microscope using a 20 × 0.75 numerical aperture objective. Images per area were captured under two colour filters achieved by a SPOT RT KE colour three-shot CCD camera (Diagnostic Instruments Inc., MI, USA), illuminated by a 170 W Xenon light source. Texas red (540–580 nm) excitation filter was used to capture MHC I images and FITC (465– 495 nm) excitation filter was used to capture MHC II and Laminin. Microscope slides were prepared such that each slide contained two serial sections from one individual from each of the included groups. Images were captured, and measured, such that ~ 600-1000 muscle fibres were included for analysis from each group. All viable muscle fibres in any particular image, excluding those displaying freeze fracture artefact and any longitudinal fibres (assessed as those with a circularity of <0.6), were included for analysis and were analysed using Image J Fiji (Schindelin et al., 2012).

ii) Myonuclear domain*:* This analysis was carried out on a subset of participants (YC; *n*=10: MA; *n*=12: OC; *n*=11). Briefly, tissue sections were fixed in 4% paraformaldehyde (PFA) for 10 min, washed 3 x 5 min in PBST, blocked for 60 min at RT (in PBS containing 2% bovine serum albumin, 5% FBS, 0.2% Triton x-100, 0.1% NaAzide, and 2% goat serum). Sections were incubated in primary antibody cocktail; MHC I (DSHB, A4.951 slow isoform; neat) and MHC II (Abcam, Cambridge, MA, USA ab91506, 1:1000) at RT overnight as previously described. Slides were washed in 3 x 5 min in PBST and incubated in the secondary antibody as previously described. Nuclei were labelled with DAPI (49,6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada), prior to cover slipping with DAKO fluorescent mounting media (Burlington, ON, Can). Slides were visualised with the Nikon Eclipse Ti Microscope (Nikon Instruments, Inc., Melville, NY, USA), equipped with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments). Images were captured and analysed using the Nikon NIS Elements AR 3.2 software (Nikon Instruments). All images were obtained on the x20 objective.

iii) Myofibre satellite cell analysis*:* This analysis was carried out on a subset of participants (YC; *n*=10: MA; *n*=11: OC; *n*=10). Muscle sections were cut at 5µm in the cryostat at 20°C. Muscle sections were fixed for 10 min in PFA followed by 3 x 5 min wash in PBS. Sections were covered for 90 mins in blocking solution (2% bovina serum albumin, 5% foetal bovine serum, 0.2% Triton X-100, 0.1% NaAzide, and 5% goat serum), then incubated overnight in primary antibodies specific for Pax7 (neat Pax7) (DSHB, USA) at 4°C overnight. Sections were incubated in appropriate secondary antibodies and re-fixed and re-blocked prior to incubation in primary antibodies specific

for laminin (ab11575; 1:500) (Abcam, Cambridge, UK) and MHC I, (BAF8) (DSHB, USA). slides were washed treated with appropriate secondary antibodies. Nuclei were labelled with DAPI (49,6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada), prior to cover slipping with DAKO fluorescent mounting media (Burlington, ON, Can). Slides were viewed and images captured as described in the myonuclear domain section.

iv) Myofibre capillary analysis: This analysis was carried out on a subset of participants (YC; *n*=10: MA; *n*=12: OC; *n*=11). Muscle sections were fixed and blocked as previously described. Muscle sections were incubated in the primary antibody CD31 (Abcam ab28364, 1:30) at 4°C overnight. Following washes, sections were then incubated in the appropriate secondary antibodies. Sections were then again re-blocked in 10% GS in PBS and incubated sequentially in the third primary antibodies, a primary antibody cocktail consisting of MHC I, MHC II and laminin. Sections were then incubated in secondary antibody cocktail, nuclei were labelled with DAPI (49,6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada) and imaged as described above.

Calculations and Measurements: Myonuclear domain was determined as the fibre area (μm^2) per nucleus and the number of nuclei per muscle fibre cross section. Quantification of (i) capillary contacts (CC, the number of capillaries around a fibre), (ii) the capillary-to-fibre ratio on an individual fibre basis (C/Fi), (iii) the number of fibres sharing each capillary (i.e., the sharing factor), and (iv) the capillary-to-fibre perimeter exchange index (CFPE index) was based on previous published protocols (Hepple et al., 1997a, Hepple, 1997).

Statistical Analysis: Data were expressed as mean ± standard deviation (SD). Normality of data was assessed using the Shapiro-Wilk test. Between group differences were identified via one-way ANOVA analysis (with group as the between-subject factor). Muscle morphological data were analysed via mixed-design ANOVA (with group as the between subject factor and fibre type as the within subject factors). Bonferroni correction was applied for multiple comparisons. Bonferroni correction was applied to correct for multiple comparisons. Significance level was set at $P < 0.05$. All calculations were performed using SPSS version 22.0 (Chicago, IL).

3.4. RESULTS

Participant Characteristics: Participant anthropometric and training characteristics are detailed in Table 1. YC were taller than MA (P=0.031). MA had lower whole-body fat mass (P=0.011), a lower body fat percentage (P=0.002) and a greater skeletal muscle index (P=0.001) than OC. YC had significantly greater appendicular lean mass than OC (P=0.03). YC and MA had significantly lower fasting glucose concentrations than OC (P=0.005 and P=0.009, respectively). MA had significantly lower serum insulin (P=0.048 and P=0.041) and HOMA-IR (P=0.03 and P=0.003) than YC and OC, respectively. There were no other significant differences in anthropometric characteristics between the groups.

	YC (N=15)	MA (N=14)	OC (N=12)
Age (years)	20.0 ± 2.7**##	67.1 ± 6.4	69.8 ± 4.1
Height (m)	1.80 ± 0.04*	1.70 ± 0.06	1.80 ± 0.07
Body mass (kg)	78.9 ± 13.3	68.7 ± 6.6	77.5 ± 14.2
BMI ($kg \cdot m^{-2}$)	24.6 ± 3.6	23.0 ± 2.0	24.5 ± 3.8
Whole-body FFM (kg)	56.9 ± 6.6	52.2 ± 3.5	52.9 ± 7.8
Whole-body FM (kg)	17.6 ± 7.4	13.3 ± 3.9#	20.9 ± 7.1
Body fat (%)	22.0 ± 5.5	19.2 ± 4.1##	26.8 ± 5.4
Skeletal Muscle Index (%)	74.0 ± 5.2	77.0 ± 4.0##	69.8 ± 5.1
ALM/Height^2 ($kg.m^{-2}$)	8.18 ± 0.79#	7.71 ± 0.54	7.39 ± 0.95
Systolic Blood Pressure (mmHg)	126 ± 9	125 ± 7	137 ± 18
Diastolic Blood Pressure (mmHg)	64 ± 8	76 ± 7	83 ± 11
Fasting Plasma Glucose ($mmol \cdot L^{-1}$)	5.33 ± 0.39##	5.35 ± 0.63##	5.96 ± 0.35
Fasting Serum Insulin (μIU/mL)	10.95 ± 3.24*	7.21 ± 3.10	11.42 ± 4.38*
HOMA-IR	2.59 ± 0.75**	1.65 ± 0.61	3.05 ± 1.16**
CRP ($mg \cdot L^{-1}$)	1.71 ± 1.89	0.77 ± 0.43	1.10 ± 0.53
Training Experience (years)	-	36.5 ± 8.1	-
Training Frequency ($sessions \cdot week^{-1}$)	-	4.5 ± 1.4	-
Training Duration ($hrs \cdot week^{-1}$)	-	7.6 ± 4.7	-
Training Distance ($km \cdot week^{-1}$)	-	210±112 / 50±15	-

*Table 4. Participant anthropometric and training characteristics Data presented as mean ± SD. FFM; fat-free mass, FM; fat mass, ALM; appendicular lean mass. Training Distance separated into cyclists (left) and runners (right). * Indicates significantly different from MA, P<0.05. ** Indicates significantly different from MA, P<0.01. # Significantly different from OC, P<0.05. ## Significantly different from OC, P<0.01.*

Physical Function: Physical function characteristics are detailed in table 2. There were no significant differences between groups for any of the SPPB component nor total scores or handgrip strength. YC produced significantly greater knee extension MVC than MA and OC (P=0.006 and P<0.001, respectively) and significantly greater elbow flexion than MA (P<0.001). YC and MA had significantly greater $\dot{V}O_{2max}$ than OC (P<0.001).

	YC (N=15)	**MA (N=14)**	**OC (N=12)**
SPPB Standing Balance (s)	10.0 ± 0	10.0 ± 0	10.0 ± 0
SPPB Semi-Tandem (s)	10.0 ± 0	10.0 ± 0	10.0 ± 0
SPPB Full Tandem (s)	10.0 ± 0	9.7 ± 1.0	9.7 ± 1.1
SPPB 3m Walk (s)	2.37 ± 0.34	2.43 ± 0.28	2.45 ± 0.44
SPPB 5x Sit-to-stand (s)	7.27 ± 1.34	7.97 ± 1.88	7.88 ± 2.03
Handgrip Strength (kg)	50.9 ± 7.6	47.0 ± 5.9	46.3 ± 8.4
Knee Extension MVC (N)	638.3 ± 82.4**##	520.4 ± 105.3	471.4 ± 117.9
Elbow Flexion MVC (N)	292.9 ± 48.6**	223.0 ± 42.8	256.8 ± 49.2
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	53.2 ± 7.3##	49.3 ± 3.6##	36.7 ± 6.5

*Table 5. Physical function and strength Data presented as mean ± SD. * Indicates significantly different from MA, P<0.05. ** Indicates significantly different from MA, P<0.01. # Indicates significantly different from OC, P<0.05. ## Indicates significantly different from OC, P<0.01. SPPB, short physical performance battery. MVC, maximal voluntary contraction. Nm, Newton metres.*

Muscle architecture: Muscle architecture results are detailed in Table 3. YC had significantly greater MT than OC for *Vastus lateralis* ($P<0.001$), *Vastus intermedius* ($P=0.002$), *Rectus femoris* ($P<0.001$) and greater MT than MA for *Rectus femoris* ($P<0.001$). YC had significantly greater θ than OC for *Vastus lateralis* ($P=0.007$), *Vastus intermedius* ($P=0.014$), *Rectus femoris* ($P=0.002$) and significantly greater θ than MA for *Rectus femoris* ($P=0.04$). MA had significantly greater L_f than OC for *Gastrocnemius medialis* ($P=0.032$) and YC had significantly greater L_f than OC ($P=0.024$) and MA ($P=0.041$) for *Gastrocnemius lateralis*.

Habitual Dietary Intake and Physical Activity: Dietary intake results are detailed in Table 4 (upper). YC total energy ($P=0.014$), fat ($P=0.019$), protein ($P<0.001$), relative fat ($P=0.044$) and relative protein intake ($P=0.005$) were greater than OC. Whereas, MA total energy ($P=0.003$), carbohydrate ($P=0.014$), relative carbohydrate ($P=0.008$), relative fat ($P<0.007$) and relative protein intake ($P=0.029$) were greater than OC. No differences in dietary intake were observed between YC and MA. Habitual activity can be found in Table 4 (lower). MA carried out significantly more absolute and relative vigorous activity than both YC ($P<0.001$) and OC ($P<0.001$). There were no other differences identified between groups.

Muscle	Architectural Feature	YC (N=15)	MA (N=14)	OC (N=12)
Vastus Lateralis	Thickness (mm)	25.7 ± 3.6$^{\#\#}$	21.7 ± 5.5	18.2 ± 4.5
	Pennation Angle (°)	17.9 ± 3.8$^{\#\#}$	14.5 ± 4.5	12.4 ± 4.7
	Fascicle Length (mm)	84.4 ± 14.3	85.5 ± 17.7	73.4 ± 17.9
Vastus Intermedius	Thickness (mm)	18.6 ± 3.5$^{\#\#}$	16.6 ± 2.7	14.0 ± 3.4
	Pennation Angle (°)	14.9 ± 4.4$^{\#\#}$	13.1 ± 2.0	11.0 ± 2.5
	Fascicle Length (mm)	74.8 ± 20.7	73.9 ± 12.4	71.4 ± 22.1
Rectus Femoris	Thickness (mm)	26.3 ± 3.5**$^{\#\#}$	19.7 ± 3.0	17.6 ± 5.0
	Pennation Angle (°)	15.6 ± 3.1*$^{\#\#}$	12.9 ± 2.7	11.6 ± 2.4
	Fascicle Length (mm)	95.5 ± 14.0	88.2 ± 13.4	83.5 ± 12.1
Tibialis Anterior	Thickness (mm)	30.2 ± 3.5	28.8 ± 3.7	30.9 ± 3.1
	Pennation Angle (°)	13.6 ± 1.6	15.8 ± 3.2	15.1 ± 2.6
	Fascicle Length (mm)	79.3 ± 14.7	69.4 ± 13.6	74.8 ± 14.1
Gastrocnemius Medialis	Thickness (mm)	17.5 ± 4.1	16.3 ± 3.1	15.0 ± 4.0
	Pennation Angle (°)	25.5 ± 4.5	23.5 ± 5.2	24.4 ± 6.3
	Fascicle Length (mm)	42.8 ± 8.8	44.7 ± 8.9$^{\#}$	35.4 ± 8.0
Gastrocnemius Lateralis	Thickness (mm)	15.5 ± 2.9	13.5 ± 3.7	13.6 ± 4.5
	Pennation Angle (°)	14.7 ± 4.3	16.9 ± 5.4	18.1 ± 4.2
	Fascicle Length (mm)	61.3 ± 14.6*$^{\#\#}$	49.1 ± 11.7	47.2 ± 10.1
Bicep Brachii	Thickness (mm)	34.7 ± 5.4	31.3 ± 4.0	33.4 ± 4.5

*Table 6. Muscle Architecture characteristics. Data presented as mean ± SD. * Indicates significantly different from MA, P<0.05. ** Indicates significantly different from MA, P<0.01. # Indicates significantly different from OC, P<0.05. ## Indicates significantly different from OC, P<0.01. Data on fascicle length and pennation angle for biceps brachii were unavailable.*

	YC (N=15)	MA (N=14)	OC (N=12)
Total Energy (kcal·day^{-1})	2700 ± 546$^{\#}$	2820 ± 437$^{\#\#}$	2113 ± 568
Total Carbohydrate (g·day^{-1})	303 ± 85	317 ± 51$^{\#}$	230 ± 84
Total Fat (g·day^{-1})	97 ± 17$^{\#}$	93 ± 19	73 ± 28
Total Protein (g·day^{-1})	122 ± 27$^{\#\#}$	100 ± 22	83 ± 20
Relative Carbohydrate (g·kg^{-1}·day^{-1})	4.0 ± 1.6	4.6 ± 0.7$^{\#\#}$	3.0 ± 1.2
Relative Fat (g.kg^{-1}·day^{-1})	1.3 ± 0.3$^{\#}$	1.4 ± 0.3$^{\#\#}$	0.9 ± 0.4
Relative Protein (g·kg^{-1}·day^{-1})	1.6 ± 0.4$^{\#\#}$	1.5 ± 0.4$^{\#}$	1.1 ± 0.3
Sedentary Activity (mins·day^{-1})	551 ± 134	585 ± 60	586 ± 129
Light Activity (mins·day^{-1})	64 ± 25	90 ± 35	85 ± 22
Moderate Activity (mins·day^{-1})	148 ± 76	148 ± 44	164 ± 70
Vigorous Activity (mins·day^{-1})	13 ± 14**	49 ± 29	4 ± 6**
Sedentary Activity (%·day^{-1})	77.5 ±27.1	67.2 ± 6.7	81.2 ±45.3
Light Activity (%·day^{-1})	8.4 ± 3.1	10.1 ± 3.6	10.2 ± 2.6
Moderate Activity (%·day^{-1})	22.2 ±19.4	16.9 ± 4.2	20.3 ± 8.4
Vigorous Activity (%·day^{-1})	1.7 ±1.5**	5.6 ± 3.2	1.1 ± 2.6**

*Table 7. Habitual dietary intake (upper) and physical activity (lower) Data presented as mean ± SD. * Indicates significantly different from MA, P<0.05. ** Indicates significantly different from MA, P<0.01. # Indicates significantly different from OC, P<0.05. ## Indicates significantly different from OC, P<0.01.*

Muscle fibre properties: Muscle fibre data are detailed in Figure 9 A-E. MA displayed larger Type I fibre CSA than OC (P=0.031), whereas YC had significantly larger Type II fibre CSA than OC (P=0.008). MA (57 ± 15 %) had a significantly greater proportion of Type I muscle fibres compared with YC (35 ± 10 %, P<0.001) and OC (41 ±10 %, P=0.008). MA (43 ± 15 %) had a significantly lower proportion of Type II fibres compared with YC (65 ± 10 %, P<0.001) and OC (58 ± 10%, P=0.012).

Myonuclear domain: Muscle fibre data are detailed in Figure 10 A-E**.** No significant differences were observed between any of the groups for Type I fibre myonuclear domain. YC had significantly greater Type II fibre specific myonuclear domain than MA (P=0.018) and OC (P=0.006). No significant differences were observed between the groups for nuclei per Type II fibre. MA had significantly more nuclei per Type I myofibre than OC (P=0.021).

Capillarisation and satellite cells: Capillarisation and SC data are detailed in Figure 11 A-G and 12 A-C, respectively. MA had significantly more capillary contacts (CC) per Type I myofibre compared with YC and OC (P=0.002 and P<0.001, respectively). MA had more CC per Type II fibre compared with OC (P=0.006). Type I capillary-to-fibre ratio (C/Fi) was greater in MA compared with YC and OC (P=0.001 and P<0.001, respectively). MA had a greater Type II C/Fi ratio than OC (P=0.004). Capillary fibre perimeter exchange (CFPE) index was greater in MA compared with YC and OC in both Type I (P=0.002 and P<0.003, respectively) and Type II fibres (P=0.002 and P<0.003, respectively). No significant differences were observed between groups for fibre type SC content.

Morphological Feature	Fibre Type	YC (N=15)	MA (N=14)	OC (N=12)
Myonuclear Domain	Type I	1034 ± 166	930 ± 145	933 ± 126
	Type II	1276 ± 230*##	1001 ± 209	953 ± 215
Nuclei / Fibre	Type I	4.19 ± 1.11	4.63 ± 0.57#	3.72 ± 0.48
	Type II	3.71 ± 0.83	3.90 ± 0.35	3.49 ± 0.41
Capillary Contacts	Type I	4.64 ± 0.72	6.21 ± 1.29††##	4.52 ± 0.73
	Type II	4.82 ± 0.94	5.50 ± 1.08##	4.19 ± 0.71
Capillary to Fibre Ratio	Type I	1.75 ± 0.32	2.52 ± 0.62†† ##	1.71 ± 0.33
	Type II	1.81 ± 0.41	2.20 ± 0.50##	1.59 ± 0.32
Capillary to Fibre Perimeter Exchange	Type I	6.94 ± 0.71	9.23 ± 1.96†† ##	7.06 ± 1.13
	Type II	6.43 ± 0.99	8.48 ± 1.58††##	6.53 ± 1.11
Satellite Cells / Fibre	Type I	0.071 ± 0.029	0.095 ± 0.059	0.056 ± 0.022
	Type II	0.077 ± 0.031	0.057 ± 0.051	0.045 ± 0.032

*Table 8. Muscle morphological features. Data presented as mean ± SD. * Indicates significantly different from MA, P<0.05. ** Indicates significantly different from MA, P<0.01. # Indicates significantly different from OC, P<0.05. ## Indicates significantly different from OC, P<0.01. † Indicates significantly different from YC, P<0.05. †† Indicates significantly different from YC, P<0.01.*

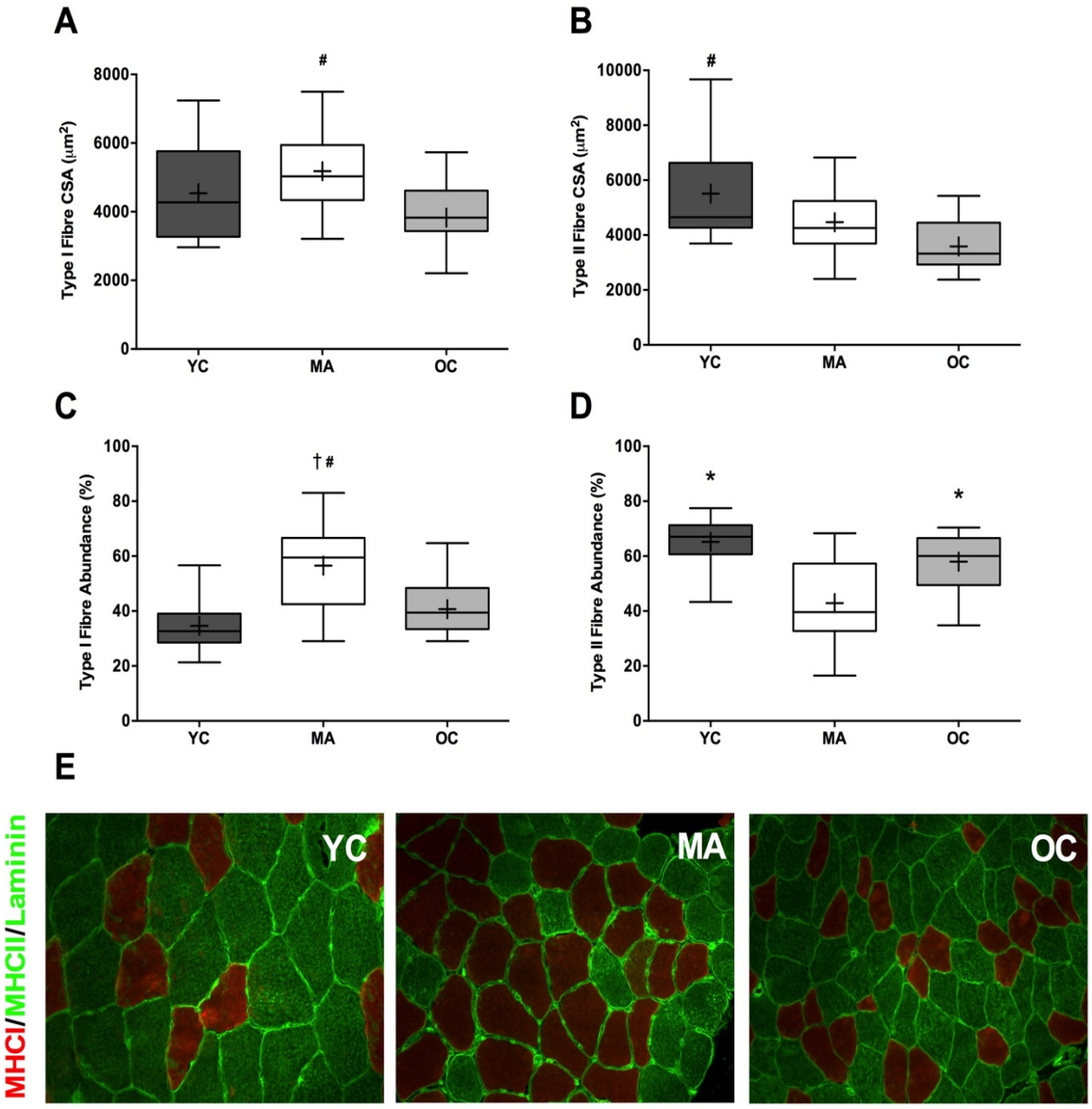

*Figure 9. Cross-sectional area (CSA) of Type I (A) and Type II muscle fibres (B) and relative abundance of Type I (C) and Type II muscle fibres (D) in young untrained individuals (YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). Representative immunohistochemical image of muscle fibre CSA from a biopsy sample in YC (left), MA (centre) and OC (right) with Type I fibre MHC in red, Type II fibre MHC in green and laminin stained cell membrane in green (E). Significance was set at P < 0.05. # indicates significantly different from OC (P < 0.05), † indicates significantly different from YC (P < 0.05) and * indicates significantly different from MA (P < 0.05). Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).*

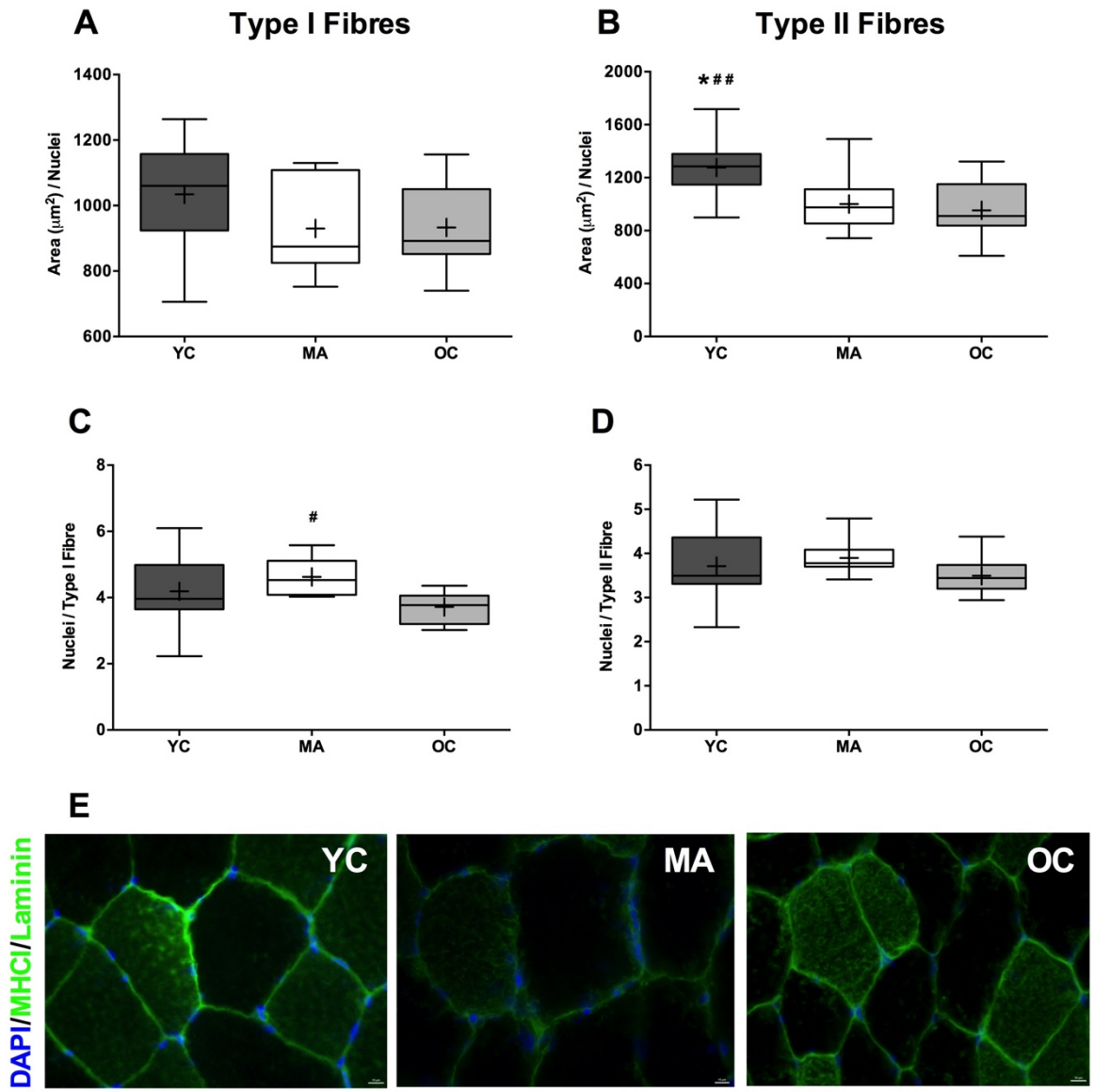

*Figure 10. Myonuclear domain in Type I (A) and Type II muscle fibres (B) and number of nuclei per Type I (C) and Type II muscle fibre (D) in young untrained individuals (YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). Representative immunohistochemical image of fibre-type myonuclei from a biopsy sample in YC (left), MA (centre) and OC (right) with DAPI stained nuclei in blue, Type I fibre MHC in green and laminin stained cell membrane in green (E). Significance was set at P < 0.05. # indicates significantly different from OC (# P < 0.05 and ## P < 0.01), * indicates significantly different from MA (P < 0.05). Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).*

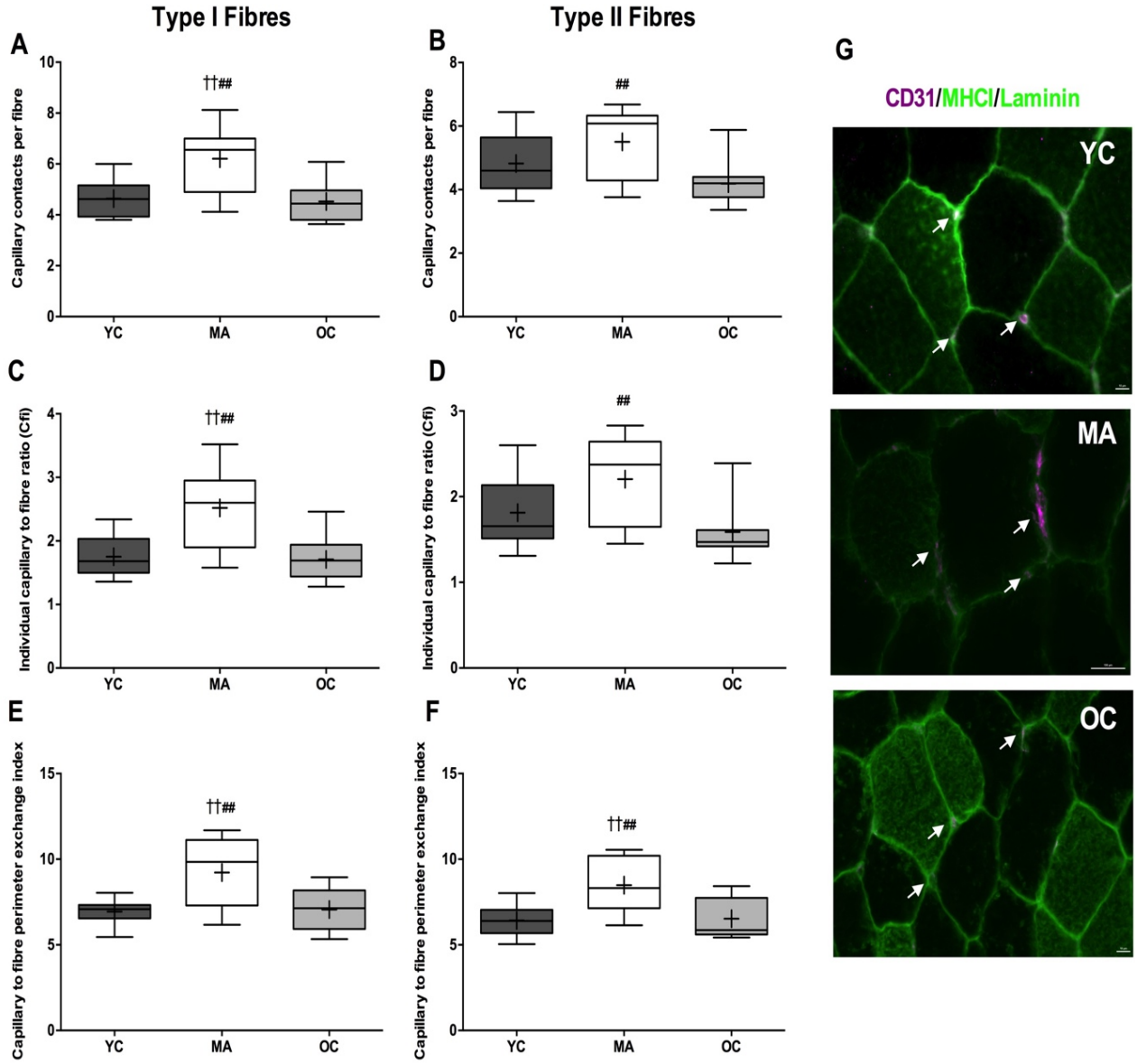

Figure 11. Capillary contacts per fibre (CC), capillary to fibre ratio (Cfi) and capillary to fibre perimeter exchange index in Type I fibres (A, C and E, respectively) and Type II fibres (B, D and F, respectively), in young untrained individuals (YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). Representative immunohistochemical image of fibre-type capillarisation from a biopsy sample in YC (top), MA (middle) and OC (bottom) with CD31 stained capillaries in purple (denoted by white arrows), Type I fibre MHC stained in green and laminin stained cell membrane in green (G). Significance was set at $P < 0.05$. ## indicates significantly different from OC ($P < 0.01$), †† indicates significantly different from MA ($P < 0.01$). Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).

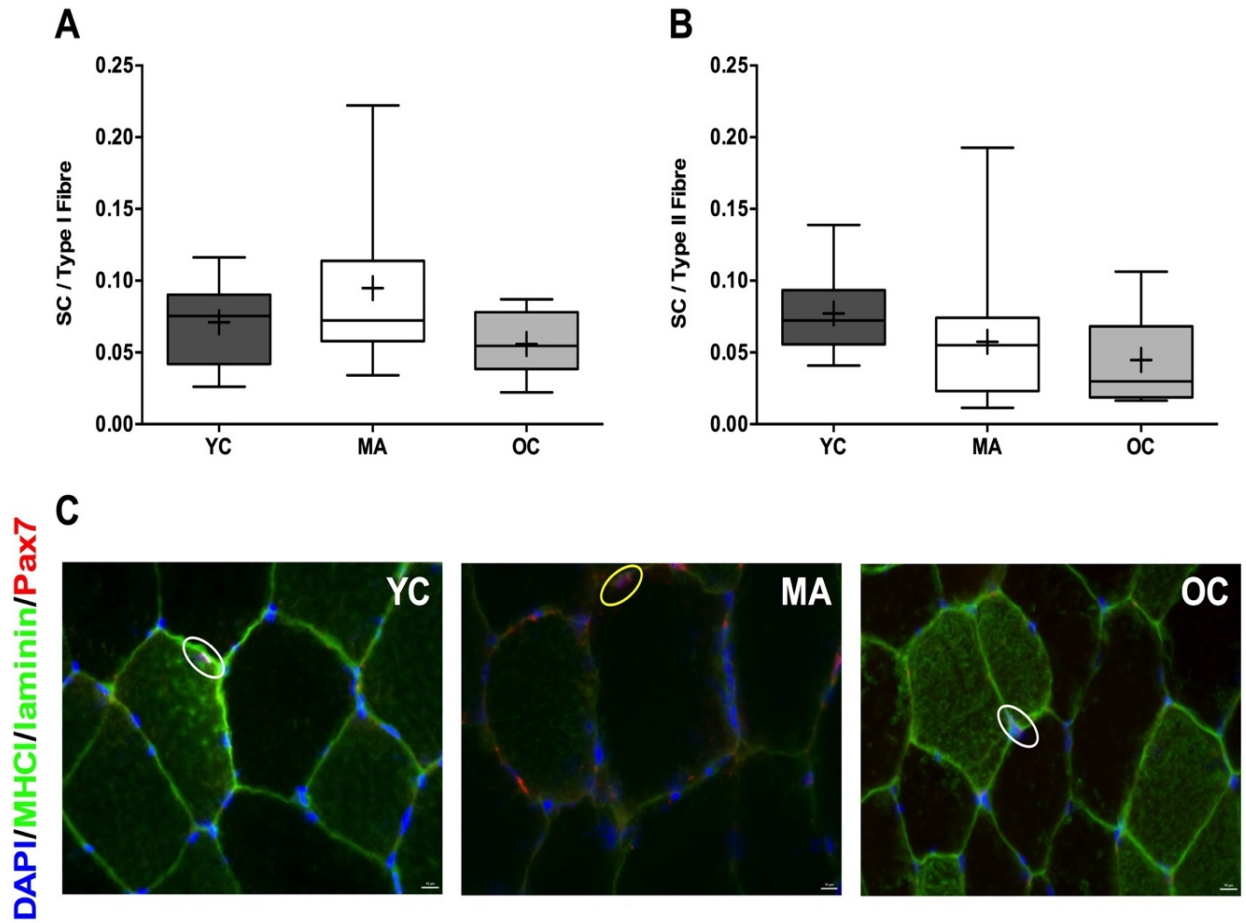

Figure 12. Satellite cell (SC) number per Type I (A) and Type II muscle fibre (B), in young untrained individuals (YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). in young untrained individuals (YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). Representative immunohistohemical image of fibre-type SC from a biopsy sample in YC (left), MA (centre) and OC (right) with DAPI stained nuclei in blue, Type I fibre MHC stained in green, laminin stained cell membrane in green and Pax7 stained SC in red (C). Circles represent SC (Pax7) overlaid with nuclei (DAPI). White circles represent SC associated with a Type I fibre (MHCI positive), yellow circles represent SC associated with a Type II fibre (MHCI negative). Significance was set at $P < 0.05$. Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).

3.5. DISCUSSION

Ageing has a deleterious effect on physiological function, muscle mass, and strength (sarcopenia) and muscle oxidative capacity that may be exacerbated by low physical activity. As such, individuals who have chronically undertaken structured exercise training, or Master Athletes (MA), provide an opportunity to study mechanisms of inherent physiological ageing dissociated from inactivity (Lazarus and Harridge, 2017). Previously, we have highlighted the dearth of studies providing a comprehensive characterisation of the MA phenotype (McKendry et al., 2018). Herein, we address this issue by firstly demonstrating that endurance-trained MA display superior body composition (lower body fat and higher skeletal muscle mass index) and aerobic fitness compared with untrained age-matched older individuals (OC), to a level comparable with untrained healthy young individuals (YC). Secondly, our novel morphological insights demonstrate that MA displayed larger Type I muscle fibre area than OC and no evidence of an age-related reduction in Type II fibre area compared with YC. Furthermore, MA displayed greater capillarisation of Type I and II fibres compared with OC and YC. Type II fibre myonuclear domain size was greater in YC compared with OC and MA, whereas SC content was similar between groups. Taken together, these data suggest that chronic exercise training promotes superior physiological conditioning, which may have implications for healthspan extension.

Sarcopenia is often accompanied, and in some cases masked, by increased adiposity (Baumgartner, 2000) and ectopic and visceral fat deposition (Goodpaster et al., 2000a, Smeuninx et al., 2017). Reduced muscle mass and increased body fat are independently and concomitantly associated with increased risk of metabolic disease (Lu et al., 2013), frailty (Buch et al., 2016), and mortality (Atkins et al., 2014). It is well established that exercise training leads to improvements in body composition. Thus, the

absence of sufficient physical activity in the face of surplus energy intake in older age, increases the likelihood of unfavourable changes in body composition (i.e., reduced muscle mass and increased body fat). Furthermore, periods of inactivity and disuse impair the normal muscle anabolic response to protein nutrition and accelerate the progression of sarcopenia (Breen et al., 2013). Our data demonstrate that MA had a greater skeletal muscle index and lower body fat than OC. This more favourable lean-to-fat mass ratio in MA vs. OC, in combination with high habitual activity, offers a likely explanation for the lower whole-body insulin resistance. Thus, it is clear that the continuation of structured endurance exercise training promotes a favourable body composition and likely confers significant physiological benefits.

Age-related loss of skeletal muscle mass and strength involves alterations in architectural properties and fibre-type morphology. With ageing, muscle fibre fascicle length and pennation angle decrease, indicating a loss of sarcomeres (in series and parallel) that would impair muscle force generating capacity (Narici et al., 2003). Our findings demonstrate that muscle fibre fascicle length and pennation angle properties were generally lower in OC and MA, compared with YC, with no clear differences between MA and OC. Similarly, previous studies have reported equivalent *vastus lateralis* fascicle length between young and old sprint athletes (Korhonen et al., 2006). However, fibre pennation angle and fascicle length of the *vastus intermedius* and *gastrocnemius medialis,* respectively, were greater in YC compared with OC only, with no difference between OC and MA. These data indicate that the alterations to muscle architectural properties with advancing age are not preserved with chronic endurance exercise training. The observed architectural modifications in MA and OC may be necessary to arrest the decline in force production with age-related muscle loss. In comparison with endurance exercise,

contractile loading in the form of resistance training may be required to generate sufficient mechanical tension to enhance muscle architectural properties in older individuals (Reeves et al., 2004a).

Reductions in muscle fibre number and area, specifically of Type II fibres, is a characteristic of sarcopenia (Nilwik et al., 2013). In older individuals, resistance training increases Type II fibre area (Kosek et al., 2006), whereas endurance exercise training primarily augments Type I fibre area (Harber et al., 2012). Skeletal muscle morphology data in MA are scarce, and available studies have yielded conflicting findings (Zampieri et al., 2015, Mackey et al., 2014, McKendry et al., 2018), making it difficult to fully elucidate the mechanisms through which chronic exercise enhances physiological function and healthspan. In line with previous findings (Aagaard et al., 2007b, Zampieri et al., 2015, Mosole et al., 2014), we demonstrate that endurance trained MA have larger Type I fibres than OC, and a greater proportion of Type I fibres compared with YC and OC. Compared with OC, the superior Type I fibre properties of MA suggests that their greater maximal aerobic capacity may be partly underpinned by a greater muscle oxidative potential, although we acknowledge the contribution of cardiorespiratory factors in VO_{2max}. Although there was no significant difference in Type II fibre area between MA and YC, neither was there any significant difference in MA vs. OC. However, it is worth noting that OC were very healthy, based on strict exclusion criteria, which may explain why we were unable to detect a difference in Type II fibre area compared with MA. Regardless, we are unable to conclude that chronic endurance exercise preserves Type II fibre area and due to technical limitations, we were only able to analyse two fibre types. Given evidence that ageing leads to a reduction in Type IIA fibres, an increased proportion of fibres co-expressing different MHC isoforms, and that physical activity can offset these

alterations (St‐Jean‐Pelletier et al., 2017), future investigations should seek to determine more specific and hybrid fibre-types. Nevertheless, the current data suggest that chronic endurance exercise elicits a mode-specific remodelling of muscle fibres, such that fibre area is greater (Type I) or indistinguishable (Type II) from YC.

Skeletal muscle SC, as the main source of new myonuclei, play an important regulatory role in the repair and regeneration of skeletal muscle (Snijders et al., 2015). Given that age-related Type II fibre atrophy is accompanied by a reduction in Type II SC, and that SC content is a strong predictor of muscle fibre size in older individuals (Verdijk et al., 2007, Verdijk et al., 2010), reductions in SC may impair the capacity for muscle maintenance in old age. As such, we were surprised to find no age-related difference in fibre-specific SC content between YC and OC. That we observed no influence of chronic endurance exercise on SC is consistent with evidence that the SC pool remains constant in response to endurance-based training programmes in humans (Joanisse et al., 2015). The absence of any difference in fibre-specific SC between groups, could be due to a large within-group variability in the sub-set of samples available for analysis. In addition to SC content, others have shown that SC activity, which is critical for muscle repair and regeneration with exercise-induced damage, is delayed in older individuals (Snijders et al., 2014) and increases in response to endurance training programmes (Joanisse et al., 2015). Measurement of SC activation was beyond the scope of the current study, but could be maintained in MA and warrants further investigation. The area of the cell governed by each myonucleus (myonuclear domain) was greater in Type II fibres of YC compared with OC and MA, whereas Type I fibre myonuclear domain size was similar between groups. Taken together, these data indicate that larger Type I fibre area in MA vs. OC, occurs independently of alterations in SC content or myonuclear domain, which appear relatively

constant with ageing and chronic endurance training. Furthermore, the lower Type II fibre area in OC vs. YC was mirrored by a diminution of the myonuclear domain and we speculate preceded any change in SC content. Finally, diminution of the Type II fibre myonuclear domain in MA compared with YC, preceded any detectable reduction in Type II fibre area.

Impairments in muscle perfusion and the delivery of oxygen and nutrients to skeletal muscle from nearby capillaries, may impair muscle oxidative capacity and contribute to the development of age-related muscle anabolic resistance and sarcopenia. Indeed, capillary density is reduced in older age, particularly in Type II fibres (Proctor et al., 1995), thereby impairing muscle fibre perfusion. In older muscle, the capillary-to-fibre ratio and the capillary-to-fibre perimeter exchange index is reduced, and the distance between satellite cells and the nearest capillary is greater in Type II fibres compared with younger individuals (Nederveen et al., 2016). This may have consequences for the responsiveness of SC to facilitate skeletal muscle repair and remodelling processes in response to contraction-induced muscle damage. In contrast to the findings of Nederveen et al. (2016), we did not observe any age-related reduction of indices of capillarisation, which may be reflective of the greater aerobic capacity of our cohort (~10.0 $mL \cdot kg^{-1} \cdot min^{-1}$), and our relatively small sample size. Nevertheless, it is clear from our results that chronic endurance exercise training leads to greater Type I and II fibre capillarisation in MA compared with OC and, in some cases, YC. These findings are consistent with previous evidence that the continuation of endurance training into older age maintains skeletal muscle capillarisation (Coggan et al., 1990, Proctor et al., 1995), regardless of training mode and intensity (Gries et al., 2018). However, recent evidence from a cross-sectional analysis of highly trained cyclists suggests that chronic endurance training does

not prevent a degree of decline in capillary density (Pollock et al., 2018). Nevertheless, the greater muscle fibre capillarisation observed in our cohort of endurance-trained MA may explain their larger Type I fibre area and 'youthful' aerobic fitness, alongside any notable age-related decline in Type II fibre area (Snijders et al., 2017).

Whilst our findings demonstrate superior aerobic capacity, body composition and muscle morphology in MA compared with OC, in many cases MA were indistinguishable from OC. This suggests that chronic endurance exercise is not completely protective against the age-related decline in a number of parameters. In addition, the cross-sectional nature of this study, and absence of a young endurance trained cohort precludes us from determining how chronic exercise influences the trajectory of physiological decline. Nonetheless, evidence suggests that MA still experience physiological and functional deterioration compared with their untrained age-matched counterparts (Pollock et al., 2015, Pearson et al., 2002). Therefore, according to the theory of Lazarus and Harridge (Lazarus and Harridge, 2017), chronic exercise shifts the 'set-point' from which the deterioration of physiological health, function or muscle morphology begins, but does not necessarily slow the rate of age-related deconditioning. Nonetheless, chronic exercise training appears to ostensibly compress the time spent with disease and disability closer to the natural end of life. Given the suggestion that the rate of physiological deterioration may be greater beyond the age of 75 years in MA compared with their untrained older counterparts (Lazarus and Harridge, 2017), it would be prudent to examine whether the differences in physiological function and morphology reported here are still apparent beyond 80 years of age. In our recent meta-analysis, we identified that chronic exercise training protects against the age-related loss of physiological function and adverse shifts in body composition in a mode-specific manner (McKendry et al., 2018). Consistent with

this, our findings show that chronic exercise training did not promote superior strength in MA compared with OC, and that YC were stronger than both OC and MA. Given the unquestionable potency of strength training for maintenance of muscle mass, strength and function in older age, investigations of physiological function and muscle morphology in strength trained MA will improve our understanding of the chronic health benefits of different exercise modes. Finally, given suggestions that the rate of physiological decline and impairments in muscle protein turnover may differ between older men and women (Kim et al., 2018), there is a need for further exploration of potential sex-specific differences in physiological function and muscle morphology of MA.

In conclusion, our findings demonstrate that chronically endurance trained MA display superior aerobic fitness, body composition, and indices of muscle fibre morphology compared with age-matched untrained older individuals, and display a phenotype that, in some respects, more closely resembles that of a healthy young untrained individual. These novel insights shed light on the extent to which chronic endurance training promotes physiological health and function in older age. Irrespective of whether chronic endurance exercise offsets the trajectory of sarcopenia or simply shifts the set-point from which muscle deterioration begins, the continuation of chronic exercise training appears to 'buy back' years of physiological function and health. Although the endurance trained older individuals in this study were competitive Master Athletes, this high-level of training may not be necessary to reap significant physiological function and health benefits. Instead, the incorporation of regular structured exercise training, that elicits sufficient intensity of effort, offers a viable strategy to arrest age-related physiological deterioration and likely extend healthspan.

4. Integrated Muscle Protein Synthesis Rates Are Comparable Between Endurance-Trained Master Athletes and Untrained Older Individuals

4.1 ABSTRACT

Background: An impaired muscle anabolic response to exercise and protein nutrition is thought to underpin age-related muscle loss. This muscle 'anabolic resistance' of old age may be exacerbated by aspects of biological ageing that may not be present in chronically trained older individuals, or master athletes (MA). The aim of this study was to compare basal and exercise-induced rates of integrated muscle protein synthesis (iMPS) and intracellular anabolic signaling in MA with healthy age-matched untrained individuals (OC).

Methods: In a parallel study design, iMPS rates were determined over a 48 h basal period and over 48 h following a bout of resistance exercise in 8 OC (73.5 ± 3.3 y) and 7 MA (68.9 ± 5.7 y). Serial skeletal muscle biopsies and daily saliva samples were obtained to determine iMPS rates using deuterated water (D_2O). In addition, intramuscular anabolic signalling was determined at 1 h post-exercise. Physical activity was monitored throughout and dietary intake was controlled during the iMPS measurement period.

Results: Anthropometric characteristics, habitual daily activity and dietary intake were similar between groups ($P>0.05$). There was no difference in basal rates of iMPS between OC (1.47 ± 0.06%·day^{-1}) and MA (1.46 ± 0.08%·day^{-1}). Resistance exercise significantly increased iMPS in both OC (1.61 ± 0.08%·day^{-1}, $P<0.01$) and MA (1.61 + 0.08%·day^{-1}, $P<0.01$), with no difference between groups ($P>0.05$). Akt^{Thr308} phosphorylation was significantly increased at 1h post-exercise in OC ($P<0.05$), but not MA ($P>0.05$). No other differences in intramuscular anabolic signalling were apparent between groups ($P>0.05$) .

Conclusions: Overall these data suggest that basal and exercise-induced iMPS are indistinguishable between MA and OC. Importantly, the OC retain a capacity for exercise-induced stimulation of skeletal muscle remodelling.

4.2 INTRODUCTION

The rapidly expanding ageing population presents a substantial concern amongst healthcare professionals and researchers alike. By the year 2050, ~23 million people will be aged 60 years or older, accounting for >30% of the UK population (Office for National Statistics: https://www.ons.gov.uk). The overwhelming challenge is that the time spent in good health in older age (i.e. health-span) is not expanding at the same rate as average life expectancy (Seals et al., 2016). Thus, it is critical that effective strategies to minimise the gap between life- and health-span are identified. Skeletal muscle is vital for the maintenance of physical function, nutrient deposition and basal metabolism (Frontera and Ochala, 2015). Ageing leads to a progressive loss of skeletal muscle mass, which progresses at a rate of 0.5-1% per annum from the 5th decade, alongside 3-5-fold greater reductions in strength (Janssen et al., 2000, Delmonico et al., 2009). Accordingly, sarcopenia may drive the development, and progression, of many adverse health events (Biolo et al., 2014, Narici and Maffulli, 2010) and force a dependence on external healthcare services (Sonn, 1996, Pinedo-Villanueva et al., 2019).

Sarcopenia (age related loss of muscle mass and function) (Rosenberg, 1989) progression is thought to be underpinned by inherent ageing factors (i.e. hormonal changes) and aggravated by environmental and lifestyle factors (i.e. poor nutrition, obesity and reduced activity) (Narici and Maffulli, 2010, Smeuninx et al., 2017), that blunt the muscle protein synthesis (MPS) response to normally robust anabolic stimuli, such as amino acids (Breen and Phillips, 2011, Shad et al., 2016). This age-related muscle 'anabolic resistance' may be underpinned by impairments in ribosomal biogenesis and/or translational efficiency in the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway (Guillet et al., 2004, Brook et al., 2016, Kumar et al., 2009). Although

exercise is effective at enhancing muscle anabolic sensitivity (Timmerman et al., 2012, Yang et al., 2012) and augmenting muscle mass and strength in older individuals (Fiatarone et al., 1990, Frontera et al., 1988), adaptive remodelling responses are attenuated compared with younger individuals (Greig et al., 2011, Brook et al., 2016). However, chronic structured exercise training is known to alter muscle protein turnover rates in young and older individuals (Wilkinson et al., 2008b, Short et al., 2004b, Yarasheski et al., 1999). Therefore, commencing exercise training in early adulthood, and continuing this practice through middle-to-older age, may prevent the decline in muscle protein synthesis and onset of muscle anabolic resistance typically seen with advancing age.

Highly-active older individuals that have maintained structured exercise training habits (Master Athletes; MA) display superior indices of physiological function (VO_{2max} and strength), muscle morphology and more favourable body composition than their untrained age-matched counterparts (McKendry et al., 2018), which could be explained by differences in MPS. The only study to date to investigate MPS in MA, reported that highly-trained master triathletes (>50 y) displayed a lower MPS rate 72 h following a bout of downhill running compared with younger triathletes (Doering et al., 2016). However, basal MPS was not measured, which prevents firm conclusions regarding the net MPS response to the exercise stimulus. Furthermore, comparison of MA with an untrained older cohort (>60 y) is required to understand whether chronic endurance exercise modulates MPS. A recent study has demonstrated that physiological function (i.e., $VO_{2max,}$ strength) and indices of muscle morphology appear to deteriorate at a similar relative (if not absolute) rate in highly-trained MA and untrained older individuals (Pollock et al., 2015, Pollock et al., 2018, Pearson et al., 2002). However, the impact of chronic exercise

training on *in vivo* metabolic and molecular regulation of skeletal muscle mass in older age, is less clear.

Therefore, the primary aim of this study was to compare 48 h basal and exercise contraction-induced integrated MPS (iMPS) rates between MA and age-matched untrained individuals. The secondary aim was to establish the acute intramuscular signalling response to an acute bout of exercise contraction. We hypothesised that basal iMPS rates would be similar between groups, but that contraction-induced iMPS rates and intramuscular signalling responses would be greater in MA versus age-matched untrained individuals, indicative of a greater (or maintained) capacity for muscle remodelling in lifelong exercisers.

4.3 METHODS

4.3.1. Participants

Eight untrained older individual controls (OC) and 7 master endurance athletes (MA) were recruited through local advertisement at athletics and cycling clubs, the British Masters Athletics Federation and the League of Veteran Racing Cyclists. Older control participants (60-80 years) were deemed eligible for the study only if they had maintained habitual activity levels and had not previously participated in any form of structured exercise training outside of recreational activities. Master athletes (60-80 years) were included only if they self-reported maintaining continuous endurance training at least twice per week for ≥20 years preceding the study. Participant anthropometric and training characteristics are detailed in

Table 9. All participants were informed of the purpose and methodology of the study, were deemed healthy by completion of a general health questionnaire assessment,

and provided their written informed consent. Ethical approval was obtained through the East Midlands – Derby Research Ethics Committee (18/EM/0004). The study conformed to the standards set by the Declaration of Helsinki (7th version).

4.3.2. Study design

In a parallel study design, OC and MA were recruited to investigate the effects of chronic endurance training on the regulation of muscle mass in an older population (detailed in the study schematic, Figure 13). Following initial study screening and consenting, participants reported to the School of Sport, Exercise and Rehabilitation Sciences (SportExR) laboratory on four separate occasions. For each visit, participants reported to SportExR in an overnight fasted-state and were asked to refrain from caffeine consumption on the day of the trial. Further, participants were asked to refrain from strenuous physical activity and alcohol for the duration of study involvement.

Visit 1: During the initial visit, participants underwent physical anthropometry and body composition measurements, and provided a single saliva sample which was collected daily throughout study involvement. After a baseline assessment of blood pressure and body composition, participants undertook bilateral 1 repetition maximum (1RM) strength testing and familiarisation with the exercise protocol. Following this, participants were given a bolus of deuterated water (D_2O) and daily top-ups for the measurement of muscle protein synthetic rates. Participants were supplied with a pedometer to monitor their habitual daily activity.

Visit 2: Seventy-two hours following visit 1, participants provided a single saliva sample and a muscle biopsy was collected. Participants were provided with a weight maintenance food parcel, matched for total calories and macronutrient content for the subsequent 4 days to standardise dietary conditions and reduce the influence of dietary variances between individuals.

Visit 3: Forty-eight hours following visit 2, participants provide a single saliva sample and a muscle biopsy was collected to determine basal iMPS. Following this, participants completed an intense bout of leg resistance exercise, which involved performing 6 sets of 10 repetitions of leg extension exercise at 75% of their predetermined 1RM. Each set was separated by 2 mins of passive rest. Participants then rested for 1 hour before another muscle biopsy was collected to examine the acute intramuscular signalling response to the resistance exercise bout.

Visit 4: Forty-eight hours following visit 3, participants reported to SportExR to provide a final saliva and muscle biopsy sample to determine exercise-induced iMPS, after which involvement in the study was completed and participants were allowed to leave.

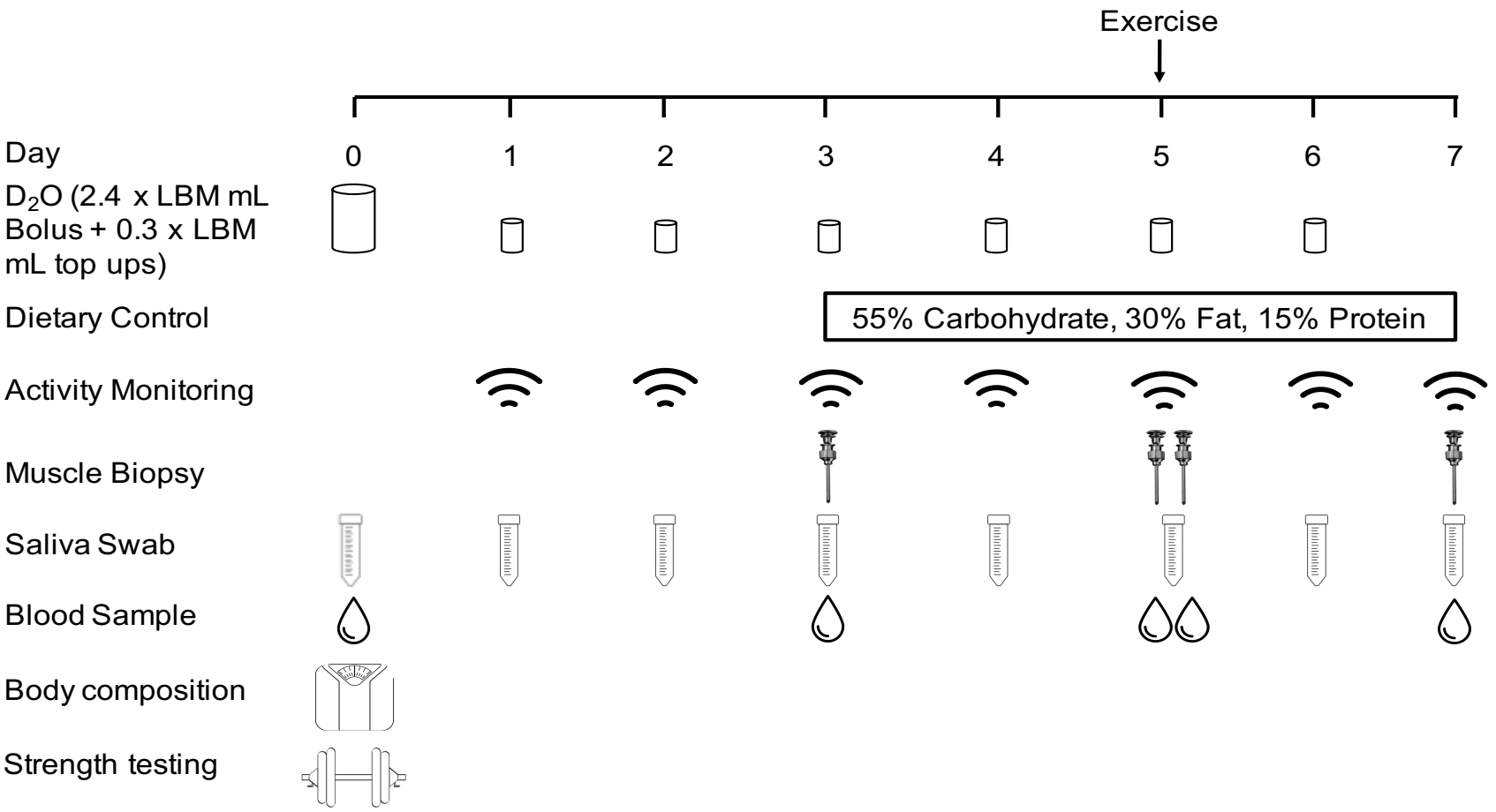

Figure 13. Schematic overview of the experimental design. LBM; lean body mass.

4.3.3. Experimental Procedures

Body mass, height and composition: Body mass was determined by weighing each participant in loose clothing, without shoes, to the nearest 0.1 kg using a digital balance scale (Seca 813, Hamburg, Germany). Height measurements were made to the nearest 0.1 cm using a stadiometer (Seca 217, Hamburg, Germany). Participants underwent bioelectrical impedance analysis in order to determine fat and fat-free mass (Bodystat QuadScan 4000, Bodystat Ltd, Isle of Man, British Isles). Individuals were instructed to lie in a supine position for ~10 mins to allow for any fluid shifts and electrodes were attached to the right-side hand and foot.

Blood Pressure: Blood pressure was measured using a standard fully automatic blood pressure monitor (OMRON M2, OMRON Healthcare UK Ltd., UK). Participants were asked to remove any clothing that obstructed the blood pressure cuff. Participants were seated with their legs uncrossed and back supported, encouraged to relax and refrain from talking during the assessment. This test was repeated 3 times and the highest reading taken.

Leg extension 1RM and exercise familiarisation: Individuals undertook 1RM bilateral knee-extension strength testing (Elite Series – Leg Extension / Curl, Fitness Warehouse Ltd, Preston, Lancashire, UK). Participants were instructed on proper lifting technique and carried out a warm up set of 10 repetitions at ~ 40% 1RM. Following which, the weight was increased and the repetitions reduced utilising a standardised 1RM testing protocol adapted from Mayhew et al. (1992) (Mayhew et al., 1992) until the participants could no longer lift the desired load, with correct form, for 1 repetition. Individuals provided

feedback on the difficulty of each set using an adapted Borg Scale (CRT-10) (Day et al., 2004, Buckley and Borg, 2011) . Participants rested for 2 mins between each set, and 3 min rest was provided between 1RM attempts. The inclusion of a resistance exercise stimulus in this study provided a novel stimulus for both OC and MA cohorts; as it has been demonstrated that chronic exercise training in young elicits a more refined, fraction specific skeletal muscle remodelling response (Tang et al., 2008, Wilkinson et al., 2008a).

Physical activity and dietary control: Participants were provided with a standard, wrist-worn, pedometer (ID115HR LETSCOM, Hong Kong) to record the average total daily step count over the duration of the study. Participants were asked to refrain from strenuous exercise for the duration of study involvement. Participants were also provided with a standardised, weigh-maintenance diet (~50% carbohydrate, ~30% fat and ~15% protein) to consume during involvement in the study. Energy requirements were calculated using the Harris-Benedict equation (Harris and Benedict, 1919). Participants could choose from a selection of pre-packaged food options, which consisted of 3 main meals (breakfast lunch and dinner), between-meal snacks and beverages. Food was provided between visit 2 and visit 4, over the course of iMPS measurement. Participants also consumed a beverage containing 20g of whey isolate protein (MyProtein, Northwich, UK), following the second biopsy on visit 3 (i.e. 1 h post-exercise) to augment exercise-induced MPS similarly in MA and OC in the initial several hours of recovery (Yang et al., 2012).

D_2O Protocol: Stable isotopically labelled D_2O was provided to participants throughout study involvement. To rapidly increase the body water enrichment of deuterium (^2H) to ~0.2%, participants consumed a bolus dose of D_2O on day 1 (0.8 x 3 x lean body mass

(LBM)). To maintain the ~0.2% enrichment of body water throughout study involvement, participants consumed a daily top dose of D_2O (0.3 x LBM). Total body water enrichment was used as a surrogate for deuterated-alanine labelling as previously described (Wilkinson et al., 2014). All doses were consumed in the morning, immediately following saliva sample provision. Saliva samples were collected in the morning, immediately on waking. Participants chewed a cotton swab for ~2 mins or until completely saturated. Samples were stored in the fridge and brought in during their next lab visit. Saliva was pressed out of the swab using a 5 mL syringe into two separate glass vials, sealed and stored at -20°C until later analysis.

Muscle Biopsy: Muscle biopsy samples were obtained from the quadriceps *vastus lateralis* under local anaesthesia (1% lidocaine) using the Bergström needle technique (Bergstrom, 1975). Muscle biopsy tissue was quickly rinsed in ice-cold saline and blotted to remove any visible fat and connective tissue before being frozen in liquid nitrogen.

4.3.4. Data analyses

Muscle protein synthesis: Muscle samples (~30-35mg) were homogenised in ice cold homogenisation buffer. Samples were centrifuged to separate the sarcoplasmic and myofibrillar fractions. The myofibrillar fraction was purified and hydrolysed to the constituent amino acids. The free amino acids were further purified on cation-exchange columns, dried and reconstituted in 0.1m HCl before analysis by gas chromatography combustion isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH). Muscle preparations were analysed for deuterated-alanine (^{2}H-alanine) with a Thermo Finnigan Delta V isotope ratio mass spectrometry coupled to a Thermo Trace GC Ultra with a gas

chromatography combustion interface III and Conflow IV. The N-acetyl n-propyl ester of alanine was analysed using a splitless injection and a Zebron ZB-5 column of 30 m × 0.25 mm × 0.50 μm film thickness (Phenomenex, Torrance, CA). The gas chromatography oven was programmed with an initial column temperature of 80°C with a 2-minute hold, followed by a ramp of 30°C min^{-1} to 330°C. Eluents were directed into the pyrolysis reactor, heated at 1450 °C, and converted to hydrogen gas (Metabolic Solutions, Nashua, NH); as described previously (Bell et al., 2015). Saliva samples were analysed for ^{2}H enrichment by cavity ring-down spectroscopy (L2130-i, Picarro Inc., California, USA). The water phase of the saliva was injected six times, and the average of the last three measurements was used for data analysis. The ^{2}H isotopic enrichments for muscle and saliva initially expressed as $\delta^{2}H‰$ were converted to atom percent excess using standard equations as previously described (Wilkinson et al., 2014).

Intramuscular signalling: Western blot analyses were performed on the sarcoplasmic fraction obtained during myofibrillar isolation (previously described (Smeuninx et al., 2018)). Sarcoplasmic protein content was determined by a DC protein assay before western blot aliquots of 2 μg protein per 1 μL were prepared in 4X Laemmli sample buffer and sucrose lysis buffer and subsequently boiled for 5 min. Equal amounts of protein (30 μg) were loaded onto 8-12.5% gels and separated by SDS-PAGE for ~1 h. Following electrophoresis, proteins were transferred onto a biotrace nitrocellulose membrane (Pall Laboratory, Portsmouth, UK.) for 1 h at 100 V. Membranes were subsequently blocked in 5% skimmed milk for 1 h and washed 3 times for 5 mins in TBST before overnight incubation at 4 ˚C in following primary antibodies (1:1000): phospho-70 kDa S6 protein kinase (p70S6K1) Thr389 (#9205), total p70S6K1(#9202), phospho-eukaryotic initiation

factor 4E binding protein (4E-BP1) Thr37/46 (#9459), total 4E-BP1 (#9452), phospho-eukaryotic elongation factor 2 (eEF2) Thr56 (#2331), total eEF2 (#2332), phospho-protein kinase B (Akt) Ser473 (#3787), total Akt (#9272), phospho-AMP activated protein kinase a (AMPKα) Thr172 (#2535), total AMPKα (#5831), phospho-p44/42 MAPK (Erk 1/2) Thr202/Tyr204 (#4370), total p44/42 MAPK (#4695), phospho-S6 Ser240/244 (#5364), total S6 (#2217), phospho-tuberous sclerosis 2 (TSC2) Thr1462 (#3611), total TSC2 (#4308) (Cell Signalling Technology, UK.). Membranes were washed 3 times for 5 min in TBST and incubated for 1 h in their respective secondary antibody (Anti-Rabbit IgG, HRP-linked Antibody (#7074)) (1:10000) and washed again 3 times for 5 min in TBST. Protein quantification was achieved by incubating the membranes for 5 min in Immobilon Western chemiluminescent HRP substrate (Merck Millipore, Watford, UK.) before being imaged using a G:BOX Chemi XT4 imager using GeneSys capture software (Syngene, Cambridge, UK.). Bands were quantified using Image Studio Lite (Li-Cor, Lincoln, Nebraska, U.S.).

Calculations: The fractional synthetic rate (FSR) of myofibrillar protein was calculated using the standard precursor-product method as described previously (Chinkes et al., 1993). In brief:

$$FSR\ (\%\ day^{-1}) = \left[\frac{(E_{Ala2} - E_{Ala1})}{E_{BW}\ x\ t}\right] x\ 3.7\ x\ 100$$

Where E_{AlaX} is the protein-bound enrichment (in atom percent excess) from muscle biopsies at time X. EBW is the mean ^{2}H enrichment (in atom percent excess) in total body water between the time points. t is the tracer incorporation time in days. Multiplication by

3.7 adjusts for the average number of ^{2}H atoms that can become incorporated into alanine, and multiplication by 100 converts the values to percentages.

Statistics: Baseline characteristics were compared using an independent samples t-test. Normality of data was assessed using the Shapiro-Wilk test. Muscle protein synthesis and intramuscular signalling were compared using a mixed-design ANOVA, one between-group factor (group) and one within group factor (time). Significance was set at $P<0.05$. Data are presented as means ± standard deviation unless otherwise indicated. All analyses were performed using SPSS version 25 for Windows (SPSS, Inc., Chicago, Illinois).

4.4 RESULTS

Participant characteristics: Participant anthropometric and training characteristics are detailed in Table 9. No significant differences were apparent in any of the baseline characteristics between the groups, excluding exercise training background.

Exercise and habitual daily activity: No significant difference was apparent between OC (92 ± 18 kg) and MA (100 ± 7 kg) in bilateral knee extension 1RM strength and total exercise volume completed (i.e., sets x repetition x load) was not difference between OC (3861 ± 581 kg) and MA (4277 ± 373 kg). The rating of perceived exertion (RPE) was not difference between OC (8 ± 2) and MA (8 ± 2). Average daily step count over the course of the study was not significantly different between OC (8386 ± 1708 steps·day^{-1}) and MA (7746 ± 1831 steps·day^{-1}).

	OC (N=8)	**MA (N=7)**
Age (years)	73.5 ± 3.3	68.9 ± 5.7
Height (m)	1.73 ± 0.06	1.76 ± 0.08
Body mass (kg)	73.8 ± 8.5	70.0 ± 4.5
BMI ($kg \cdot m^{-2}$)	24.8 ± 3.3	22.0 ± 1.6
Whole-body FFM (kg)	52.9 ± 3.6	52.0 ± 3.8
Whole-body FM (kg)	20.9 ± 6.9	16.0 ± 1.5
Body fat (%)	27.8 ± 6.7	23.6 ± 1.9
Skeletal Muscle Index (%)	72.2 ± 6.7	76.4 ± 1.9
Systolic Blood Pressure (mmHg)	134 ± 10	130 ± 8
Diastolic Blood Pressure (mmHg)	78 ± 9	81 ± 3
Training Experience (years)	-	47.7 ± 14.8
Training Frequency ($sessions.week^{-1}$)	-	4.3 ± 1.6
Training Duration ($hrs.week^{-1}$)	-	8.4 ± 6.6
Training Distance ($km.week^{-1}$)	-	180 ± 29 / 55 ±15

*Table 9. Participant anthropometric characteristics and training background. Data presented as mean ± SD. FFM; fat-free mass, FM; fat mass. * Indicates significantly different from MA, P<0.05.*

Body water ^{2}H enrichment: Body water ^{2}H enrichment is shown in Figure 14. Body water ^{2}H enrichment significantly increased in both groups ($P<0.001$) and steady-state was maintained throughout, with no significant difference between groups at any time point.

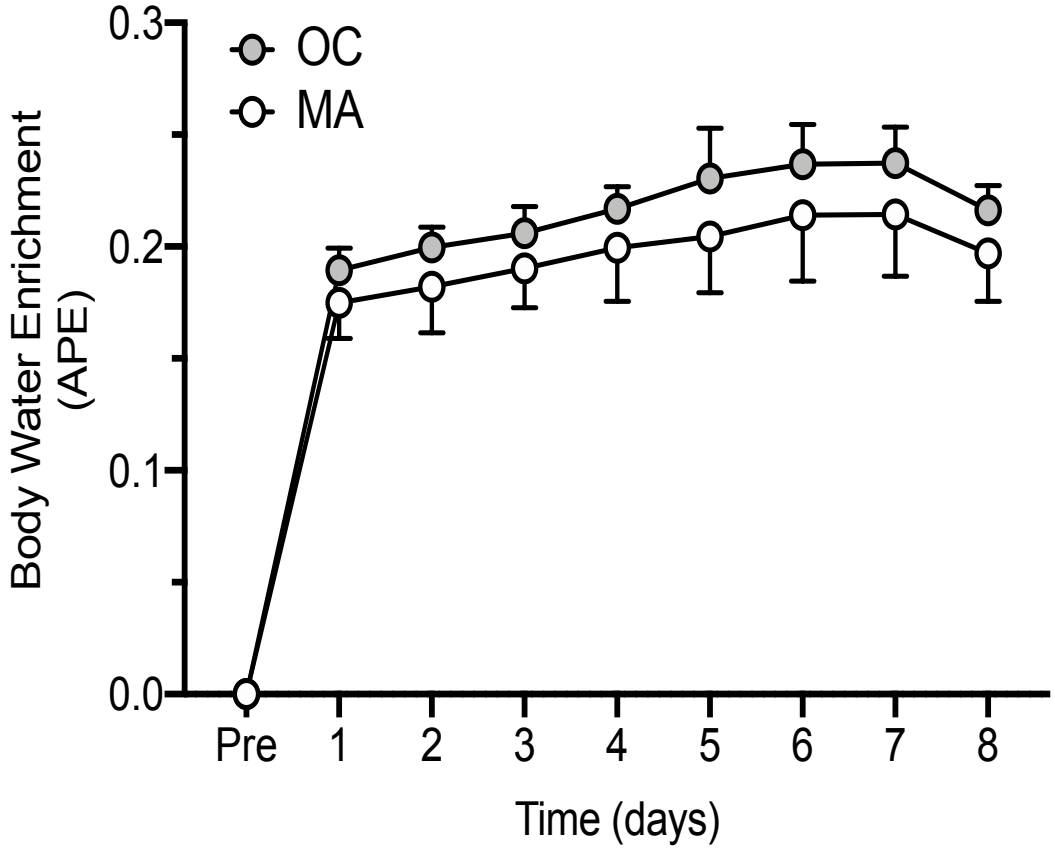

Figure 14. Body water ^{2}H enrichment (Atom Percent Excess; APE) in older untrained controls (OC, grey circles) and Master Athletes (MA, white circles). Values are presented as means ± SEM.

Myofibrillar protein synthesis: iMPS is shown in Figure 15. Rates of day-to-day iMPS were not different between OC (1.47 ± 0.064 %·day^{-1}) and MA (1.45 ± 0.078 %·day^{-1}). iMPS increased significantly in response to resistance exercise in both OC (1.60 ± 0.083 %·day^{-1}, P=0.032) and MA (1.61 ± 0.078 %·day^{-1}, P=0.034), with no significant difference between groups.

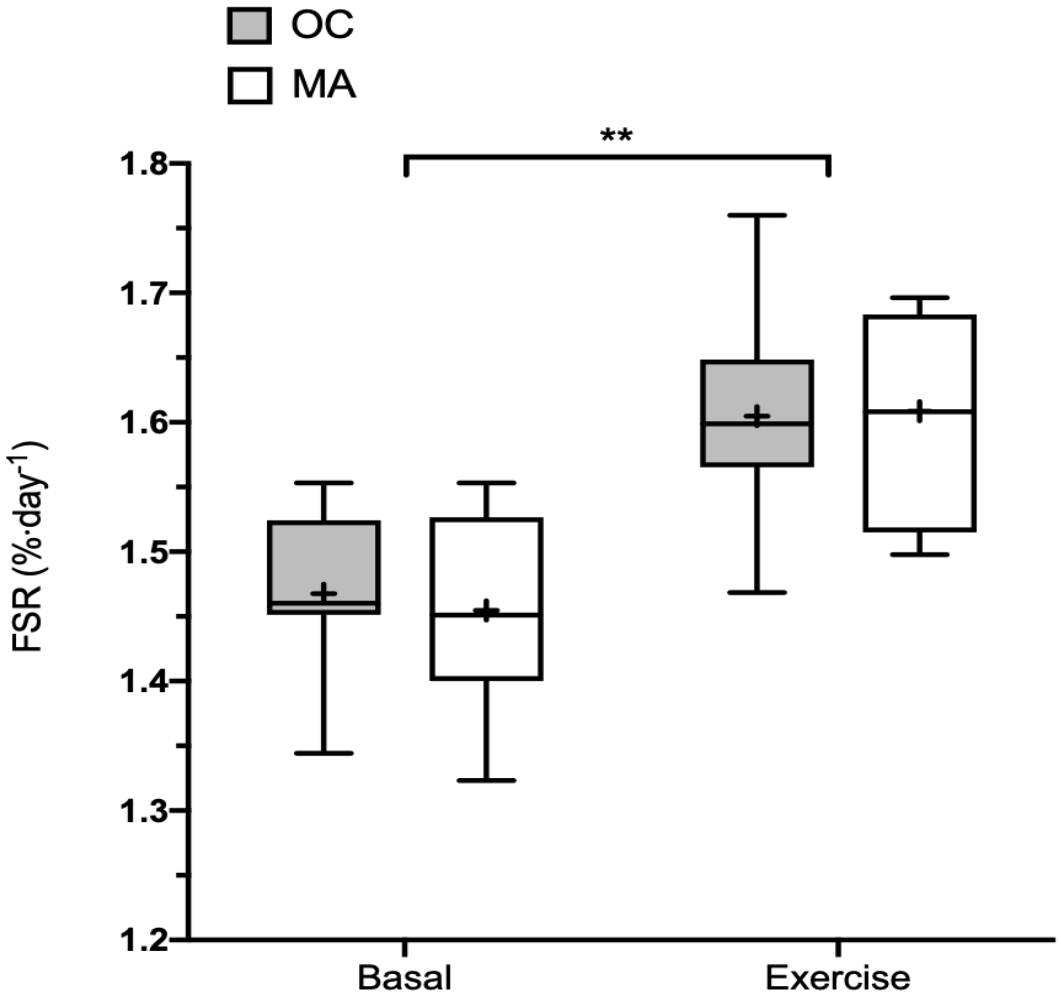

*Figure 15. 48 h Integrated myofibrillar Protein Synthesis rates in the basal state and following a bout of resistance exercise in older untrained controls (OC, grey bars) and Master Athletes (MA, white bars). ** indicates significantly different from basal values (P < 0.01). Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).*

Intramuscular anabolic signalling: Intramuscular anabolic signalling is shown in Figure 16. Phosphorylation of Akt^{Thr308} increased significantly from basal in OC only at 1 h post-exercise ($P=0.032$) and phosphorylation of Akt^{Thr308} was significantly greater in OC compared with MA at 1 h post-exercise ($P<0.05$). Phosphorylation of $p70S6K^{Thr241}$ tended to increase from basal in OC at 1 h post-exercise ($P=0.064$) with a trend for a greater phosphorylation in OC compared with MA at 1 h post-exercise ($P=0.079$). Phosphorylation of $RPS6^{Ser240/244}$ increased significantly from basal in OC ($P=0.033$) and MA ($P=0.048$) at 1 h post-exercise, with no difference between groups.

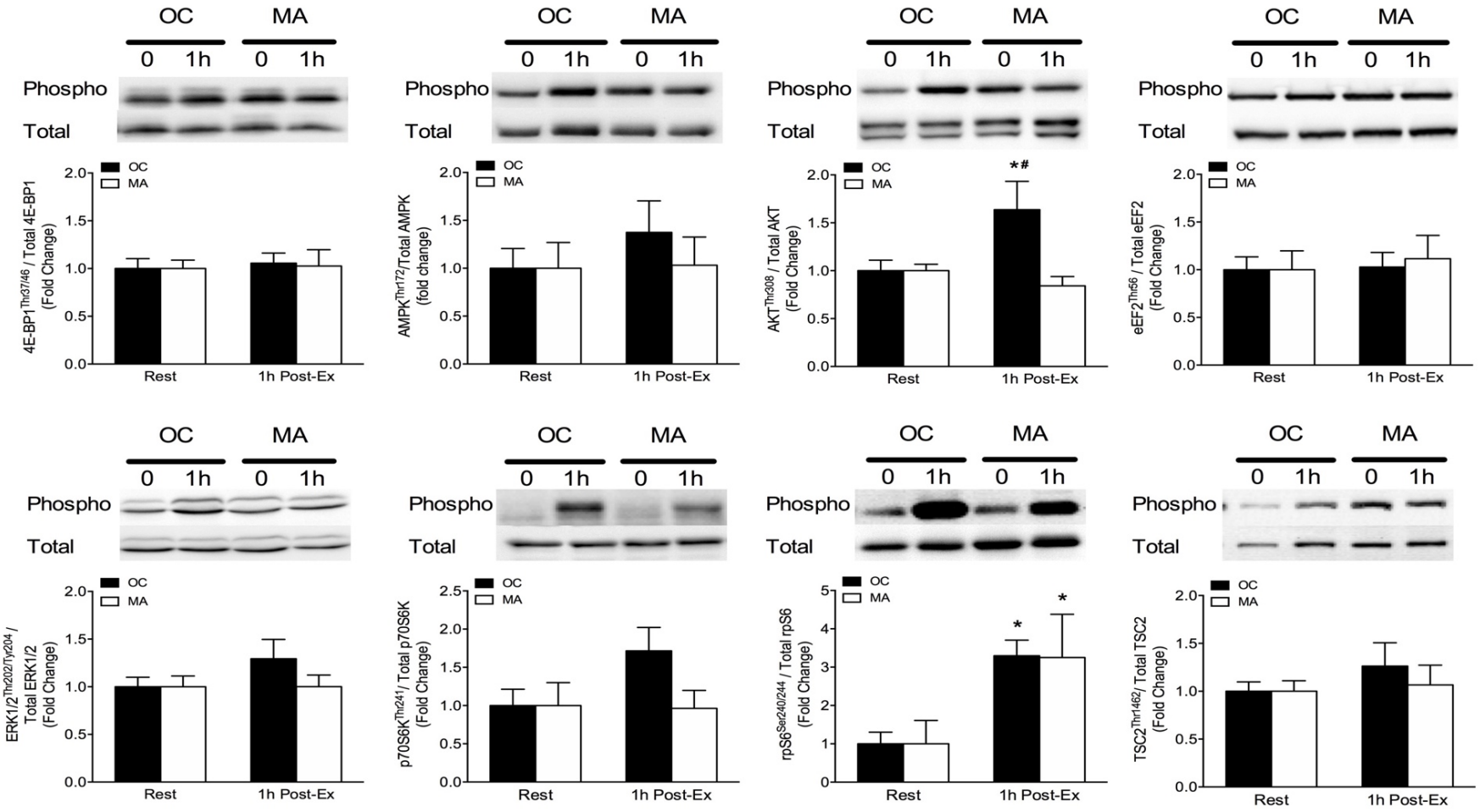

*Figure 16. Intramuscular anabolic signalling at rest and 1 h post resistance exercise in older untrained controls (OC, black bars) and Master Athletes (MA, white bars). Significance was set at P < 0.05. * indicates significantly different from basal (P < 0.05). # indicates significantly different from MA (P < 0.05). Values are presented as means ± SEM.*

4.5. DISCUSSION

A loss of skeletal muscle mass is a commonly observed consequence of ageing (sarcopenia). Anabolic resistance is often proposed as a key mechanism contributing to the development, and progression, of sarcopenia (Burd et al., 2013). Anabolic resistance is apparent with inherent ageing but thought to be exacerbated by aspects of environmental and lifestyle factors (i.e., inactivity, obesity (Smeuninx et al., 2017)). Individuals that have regularly undertaken structured exercise training (Master Athletes; MA) display superior levels of physiological function and muscle morphology compared with age-matched non-athletes (OC), and provide an opportunity to unpick the contribution of inherent and biological ageing in sarcopenia (Lazarus and Harridge, 2007). However, there are a dearth of in vivo mechanistic studies of muscle metabolic regulation in MA (McKendry et al., 2018). Here, we provide the first comparison of day-to-day iMPS rates between MA and OC under basal and exercise-induced conditions, while under strict control and monitoring of diet and activity. Our findings demonstrate no discernible difference in basal or exercise-induced iMPS rates between MA and OC. Furthermore, we highlight minor differences in the intramuscular anabolic signalling response to exercise between MA and OC (i.e., mTORC1 pathway). Taken together, these data suggest that despite divergent lifelong exercise habits, MA and OC display a similar capacity to upregulate anabolic signalling and iMPS in response to resistance exercise.

Understanding the underlying causes of sarcopenia and the development of therapeutic strategies to combat this disease are of paramount importance. Generally, physical activity declines with advancing age (Jefferis et al., 2014) and reductions in activity status (i.e., step-reduction or immobilisation) results in the development of muscle anabolic resistance and considerable muscle atrophy in young (Glover et al., 2008, Tanner

et al., 2015) and old (Breen et al., 2013, Drummond et al., 2012). Although exercise is a potent stimulus for increasing acute intramuscular anabolic signalling and MPS, this response has been found to be delayed or absent in older compared with younger individuals (Drummond et al., 2008, Kumar et al., 2009), alongside a blunted adaptive response to exercise training interventions (Greig et al., 2011, Brook et al., 2016). Importantly, our MA cohort reported consistent training habits for an average of ~50 years, and displayed superior physiological function, body composition and muscle fibre morphology compared with OC (see chapter 3) (Zampieri et al., 2015, McKendry et al., 2018). Given evidence that chronic exercise training alters basal and exercise-induced MPS responses (Wilkinson et al., 2008b, Short et al., 2004a), it is intuitive to expect that MA may demonstrate divergence in their capacity for skeletal muscle remodelling compared with OC. Surprisingly, and contrary to our initial hypothesis, basal and exercise-induced rates of iMPS were indistinguishable between MA and OC. Thus, the superior physiological function and muscle morphological properties reported in MA do not appear to be reflected by alterations to either basal or exercise-stimulated MPS rates.

The current study extends earlier work demonstrating that middle-aged master triathletes aged ~53 years have a significantly lower iMPS response to a downhill running stimulus compared with young triathletes aged ~27 years (Doering et al., 2016). The absence of a basal iMPS measurement and untrained age-matched cohort in the study of Doering and colleagues (Doering et al., 2016), precluded the authors from identifying whether advancing age and training/activity status influences the iMPS response to exercise. The incorporation of a basal measurement of iMPS in the present study demonstrates that chronic exercise does not alter the muscle anabolic responsiveness to exercise contraction in older age. Previously, no differences in basal iMPS have been

reported when comparing young and old individuals (Brook et al., 2016). In the present study, OC and MA displayed similar basal rates of iMPS, which are consistent with values reported elsewhere in older individuals (i.e., ~1.40 - 1.50%·day^{-1}) (McGlory et al., 2018). Thus, differences in physiological function and muscle morphology between MA and untrained older individuals (McKendry et al., 2018), appear not to be driven by differences in basal iMPS. It is well established that resistance exercise-induced contraction is a potent stimulus for MPS in young and old (Moore et al., 2009b, Yang et al., 2012) and that chronic exercise training can alter muscle protein turnover rates in young and older individuals (Wilkinson et al., 2008b, Short et al., 2004b, Yarasheski et al., 1999). Given the plausibility of a diminished exercise-induced iMPS with advancing age in OC (Brook et al., 2016), we expected that the iMPS response to exercise-contraction would be greater in MA vs. OC. However, our data demonstrate that OC display a similar capacity to increase iMPS rates in response to exercise contraction as MA (~10% increase over 48 h post-exercise). Despite a similar exercise-induced iMPS response between MA and OC, a likely explanation for the disparity in physiological function and muscle morphology between MA and OC, may be explained by repeated stimulation of iMPS and muscle protein accretion/maintenance over ~50 y of consistent training habits in MA. Finally, assuming similar basal iMPS rates between the present study and Doering et al. (Doering et al., 2016), the collective evidence suggests that the iMPS response to exercise is reduced with advancing age, irrespective of training status.

To gain further insight into the mechanistic regulation of iMPS, and to identify whether detectable differences in MA and OC were apparent, the phosphorylation status of a number of intramuscular signalling intermediates were measured at 1 h post-exercise contraction. We observed only subtle differences between the groups, in that Akt

phosphorylation increased by ~60% at 1 h post-exercise in OC, but not MA. Additionally, the phosphorylation of rpS6 increased two-fold in OC and MA, with no differences between groups. Unexpectedly, we did not observe a significant increase in the phosphorylation of p70S6K or 4E-BP1 in MA, and only a tendency for an increase in p70S6K phosphorylation in OC (P=0.06). It may be that the phosphorylation of signalling events that regulate MPS is delayed with ageing, regardless of training status, and that a response occurred >1 h post-exercise (Smeuninx et al., 2018, Farnfield et al., 2012, Drummond et al., 2008). Conversely, the small numbers (i.e., *n*=8 vs. *n*=7) recruited for this investigation may preclude the identification of within- and between-group differences due to the large inter-individual variability in the phosphorylation of anabolic signalling proteins. Furthermore, given that protein/amino acid feeding is required to maximize intramuscular anabolic signalling events and MPS, fasted-state post-exercise biopsy sampling in our study may have only captured a dampened signalling response. Therefore, the inclusion of additional post-exercise biopsy collection points, including the presence of protein/amino acids, may be required to provide a greater temporal resolution of the post-exercise anabolic signalling regulation in MA and OC. Regardless, the acute intramuscular phosphorylation events following exercise contraction are complex, and a discordance between anabolic signalling responses, MPS and muscle mass gain over time have been reported (Mitchell et al., 2014). As such, it is doubtful that transient phosphorylation of a single protein could explain a large degree of variation in skeletal muscle remodelling. The marginally greater degree of Akt and p70S6K phosphorylation evident in OC, could indicate that OC require a greater stimulation of the mTORC1 pathway intermediates in order to achieve a comparable iMPS response to MA; though this speculation requires further confirmation. Taken together, acute basal and exercise

contraction-induced anabolic signalling phosphorylation generally were indistinguishable between MA and OC, mirroring the equivalent iMPS rates between groups.

Age-related muscle anabolic resistance is most readily detectable in conditions of insufficient exercise volume and/or low doses of protein nutrition (Shad et al., 2016), thus mimicking typical habits of older individuals. Previous studies utilising acute intravenous infusions of stable isotope tracers have demonstrated age-related muscle anabolic resistance in response to exercise (Kumar et al., 2009) and feeding (Breen et al., 2013, Smeuninx et al., 2017). The incorporation of an orally ingested D_2O isotope tracer in the present study, enabled us to assess iMPS in free-living conditions over 48 h, unconstrained by a strictly controlled laboratory environment. Recent work has demonstrated that iMPS rates measured using D_2O closely predict muscle mass gain over time in younger individuals (Damas et al., 2016). As such, our findings provide insight into skeletal muscle remodelling that may be more reflective of changes in muscle mass over a prolonged period than previous acute studies. Specifically, the capacity for exercise-induced muscle remodelling appears to be similar between MA and OC. Nevertheless, a number of important considerations need to be addressed in future studies. Muscle protein breakdown plays an important role in skeletal muscle remodelling (Tipton et al., 2018), and it cannot be discounted that disparities exist in the rates of proteolysis between OC and MA. In addition, this study did not include a young group for comparison which prevents the identification of age-related impairments in iMPS and anabolic signalling. Finally, a RE stimulus was incorporated in this study as it presented a novel exercise challenge for both MA and OC, although it should be considered that differences in skeletal muscle remodelling may become apparent in these two cohorts when investigating fraction-

specific iMPS (i.e., chronic endurance training may preferentially modify mitochondrial protein synthesis rates) (Wilkinson et al., 2008b).

In conclusion, we have demonstrated for the first time, equivalent rates of basal and exercise contraction-induced iMPS in OC and MA, which coincided with a similar acute post-RE signalling responses between groups. Irrespective of the superior physical capabilities commonly observed in MA, OC still retain the capacity to increase iMPS in response to exercise. Furthermore, as MA regularly undertake exercise training, the repeated stimulation of adaptive remodelling responses may explain their superior physiological function and muscle morphology compared with OC. As a result, emphasis should be placed on encouraging individuals to undertake structured exercise training early in adulthood to shift the set-point from which age-related physiological deterioration begins. Importantly, this should be reflected in revised exercise guidelines, for older adults, aimed at augmenting physical function and healthspan extension.

5. General Discussion

The rapidly expanding ageing population warrants investigation into strategies to enhance age-related musculoskeletal health with a view to reducing associated healthcare costs. Muscle mass and function progressively deteriorate from middle-to-older age (termed 'sarcopenia'). Preservation of skeletal muscle mass and function are critical for those looking to progress into their later years, unburdened by disease and illness. Protracted periods of disuse (i.e., reductions in physical activity and immobilisation) and the presence of obesity, prominent characteristics in the ageing population, exacerbate the rate at which skeletal muscle is lost. The continuation of structured exercise training with ageing may offer a viable strategy for those looking to postpone the decrease in muscle mass and physiological function, and extend their healthspan. Master athletes (MA) are a unique older population that have continued to undertake structured exercise training from younger through to older age. The study of MA provides an opportunity to distinguish the relative contribution of inherent ageing and secondary lifestyle factors (i.e., inactivity, obesity) to the reductions in muscle mass and function, and establish the extent and mechanisms through which lifelong physical activity can promote healthy musculoskeletal ageing and improve quality of life. Despite the obvious significance of studying MA, research in this cohort is scarce.

Therefore, the purpose of this thesis was threefold:

i) To summarize the current knowledge-base on whether MA preserve indices of physical function and skeletal muscle properties by conducting a thorough, systematic, synthesis and evaluation of the current literature.

ii) To build upon current research by conducting a high-quality cross-sectional study, utilising sophisticated measurement techniques to comprehensively characterise physiological function, muscle mass and morphology in chronically trained MA.

iii) To investigate the metabolic regulation of skeletal muscle mass in chronically trained MA, and determine whether MA demonstrate superior skeletal muscle remodelling during basal conditions and following an exercise stimulus, and to characterise the intramuscular mechanisms coordinating this response.

This chapter provides a brief summary of the key findings from the studies reported in Chapters 2-4 of this thesis. First, based on Chapter 2, this section discusses the findings of the systematic review carried out, highlighting what has been overlooked in the research and provides recommendations for the directions of future research in MA. Second, this chapter discusses how the characterisation study carried out in Chapter 3 addresses some of the shortcomings of previous research and speculates how chronic exercise training of divergent modalities in a variety of populations may have altered the present findings. Third, Chapter 4 discusses how ageing and chronic exercise training impact skeletal muscle mass regulation. Finally, this chapter summarises the collective findings of this thesis, identifies the implications of chronic structured exercise training and makes recommendations for future research directions.

5.1. Muscle Morphology and Performance in Master Athletes

The systematic review carried out in Chapter 2 is the first qualitative and quantitative synthesis of research carried out in a MA population (McKendry et al., 2018). Importantly, this study included MA above 60 years, as typically untrained older

individuals at this life stage would have undergone some degree of muscle deterioration in skeletal muscle function, mass and morphology as a result of the canonical ageing process. The work reported in this thesis has clearly demonstrated that some, but not all, age-related decrements may be delayed with chronic structured exercise training. Specifically, this work has shown that body composition (i.e. ratio of lean to fat mass) and indices of skeletal muscle function are superior in MA compared with OC in an exercise mode-specific manner. Specifically, individuals that have carried out chronic endurance training display a higher VO_{2max} and those that have undertaken chronic strength training display greater muscular strength (McKendry et al., 2018). Poor aerobic capacity and muscular strength coupled with a sub-optimal body composition (i.e., low muscle mass and increased body fat) drastically increases the risk for the development of disease and disability (Ruiz et al., 2008, Lee et al., 2010, Ortega et al., 2012). Thus, older individuals should be encouraged to undertake structured exercise training of different modalities. In fact, the development of muscular strength, aerobic capacity and a healthy body composition through structured exercise training should be a primary focus for individuals from a young age or early adulthood. The continuation of endurance and strength training throughout the lifespan offers a viable strategy to maintain or enhance aerobic capacity and muscular strength. The physiological benefits of lifelong exercise training would therefore shift the set-point from which a loss of physiological function occurs, reducing the risk, and delaying the onset, of disability, disease and reliance on healthcare services. Consequently, functional independence would be maintained, the health-span extended and the over-all quality of life improved in chronically trained older individuals.

In Chapter 2, this work highlighted a dearth of studies utilising sophisticated measurement techniques to characterise skeletal muscle mass and morphological

characteristics in MA. Accordingly, there was no clear consensus as to whether or not chronic exercise training, regardless of modality, can protect against the canonical age-related alterations to physiological function, strength, muscle mass, architectural properties and muscle fibre morphology. Furthermore, the present work identified data for only 268 women, whereas data for 2181 men were included. Seemingly, men and women undergo distinct age-related physiological deterioration; perhaps driven by sex-specific hormonal alterations (Hansen and Kjaer, 2014, Kim et al., 2016b). Taken together, this work has identified a paucity of modality-specific, mechanistic research of men and women MA. In order to address this, future studies should adopt a more mechanistic approach, utilising appropriate measurement tools, to identify the impact of chronic exercise training on physical function, skeletal muscle mass and morphological properties.

5.2. Superior Physiological Function and Skeletal Muscle Morphology in Chronically Trained Master Endurance Athletes Compared with Untrained Older Individuals

Chapter 3 set out to address some of the shortcomings of MA-related research identified in the preceding chapter. The aim of this study was to compare physical function, cardiorespiratory fitness, body composition, muscle strength, architecture and fibre-type properties in MA, with healthy untrained younger and age-matched older individuals. As a result, the present work has shown that endurance trained MA display a superior body composition (i.e., lower body fat and higher skeletal muscle index) and aerobic fitness compared with OC, and similar to that of YC. At the architectural level, fibre pennation angle and fascicle length in MA and OC are generally lower than YC, with some exceptions (i.e. fibre pennation angle and fascicle length of the *vastus intermedius* and *gastrocnemius medialis* were greater in YC compared with OC, but not MA).

Furthermore, at the morphological level MA displayed larger Type I muscle fibre area than OC and no evidence of an age-related reduction in Type II fibre area compared with YC. Thus, in many cases, MA provide evidence that chronic structured endurance training can delay the age-related deterioration in physical function and skeletal muscle properties. However, in some cases (i.e., capillarisation) MA were superior to both OC and YC, highlighting the potency of endurance training in promoting favourable morphological alterations that likely support the superior levels of function and morphology in this cohort. In conclusion, the present work demonstrated that irrespective of whether structured exercise training alters the trajectory at which age-related deterioration occurs, and upward rightward shift of physiological function and its supporting mechanisms may be critical in extending the health-span in older individuals who undertake chronic exercise training.

Divergent exercise modes underpin the development of distinct phenotypes and functional benefits. The MA cohort included in this study had strictly undertaken chronic endurance exercise for a period of ~40 years, consecutively. Initially, this study attempted to recruit a fourth group of strength/power trained MA, however we were unable to recruit sufficient numbers of Master Power Athletes (MPA) to provide meaningful comparison. Nevertheless, it is well established that resistance exercise enhances muscular strength, muscle mass and Type II fibre CSA (Kosek et al., 2006), along with alterations to skeletal muscle architecture (Suetta et al., 2008, Reeves et al., 2004b). Thus, it could be expected that the results observed in endurance trained MA (i.e., greater VO_{2max}) would be mirrored in MPA (i.e., greater strength, muscle mass and type II fibre area than OC). Furthermore, the absence of young strength and endurance trained men and women, prevents firm conclusions on whether or not skeletal muscle parameters do decline in MA, and whether

or not sex influences this decline. The decline of men and women's world-records in almost every sporting discipline, leads one to assume that young well-trained individuals, regardless of sex or exercise training modality, would display superior physiological function and skeletal muscle morphology when compared with MA and MPA. However, it is still evident that deterioration in physiological function and skeletal muscle parameters still occurs in MA despite continuous exercise training (Pollock et al., 2015, Pearson et al., 2002). Nevertheless, structured exercise training throughout life offers an excellent strategy to shift the set point of deterioration upwards and to the right, to support optimal ageing.

This work drew attention to the scarcity of fibre-type specific satellite cell data in MA research. A reduction in satellite cell content of older muscle, specifically in Type II fibres (Snijders et al., 2009), has been demonstrated. In Chapter 3, this work has shown no difference in satellite cell content between MA, OC and YC between Type I or Type II muscle fibres or between groups. This discrepancy could be explained by the high physical function and aerobic capacity displayed in our cohort of OC. It should be considered that satellite cell content, under basal-rested conditions, may not be indicative of skeletal muscle repair capacity. Nevertheless, twelve weeks of heavy resistance exercise results in an increase in satellite cell content (Mackey et al., 2007), and aerobic exercise has been shown to increase satellite cell content, specifically in Type I muscle fibres (Fry et al., 2014). Considering these findings, our observations appear perplexing. The inclusion of an exercise stimulus and additional muscle biopsy samples would provide a more comprehensive insight into whether chronic exercise training is preventative of age-related decline in skeletal muscle repair mechanisms. However, the purpose of this study was a cross-sectional characterisation of muscle function, mass and morphological

characteristics and future studies should endeavour to delineate the potential differences in satellite cell responsiveness in these cohorts. Nevertheless, an acute exercise stimulus has been demonstrated to activate satellite cells in young men, and this response is reportedly delayed with ageing (Snijders et al., 2014) perhaps indicating older individuals require more extensive recovery periods to successfully adapt to the imposed exercise stimulus. Based on the only study to investigate the effect of chronic endurance exercise training on the satellite cell proliferative response (Murach et al., 2016), the present work led the authors to speculate that structured exercise training, over many years, may refine the adaptive response of satellite cells in skeletal muscle repair, without necessarily modulating the number of satellite cells per fibre.

The delivery of oxygen and nutrients to skeletal muscle is dependent on the adequate perfusion, underpinned by nearby capillaries. In Chapter 3, this work demonstrated that MA have a superior number of capillary contacts, capillary-to-fibre ratio and capillary-to-fibre perimeter exchange index than OC, in Type I and Type II muscle fibres. Moreover, MA demonstrate superior capillarisation of Type I and greater capillary-to-fibre perimeter exchange index in Type II fibres when compared with YC. Collectively, these data clearly demonstrate that chronic structured endurance training, over many years, is a potent stimulus to increase skeletal muscle blood supply. Interestingly, the absence of an age-related reduction in capillarisation in our hands, is likely reflective of the relatively high degree of physical function of the OC cohort in this study, when compared with previous studies (i.e., a VO_{2max} of 36.7 $ml \cdot kg^{-1} \cdot min^{-1}$ in untrained individuals of ~70 years) (Nederveen et al., 2016). The enhanced capillary network surrounding skeletal muscle in MA may play an important role in the delivery of nutrients and the activation of satellite cells to support skeletal muscle hypertrophy in

response to exercise (or offset sarcopenia) (Snijders et al., 2017). Thus, the investigation of whether MA, with a superior capillary network, are able to activate satellite cells to a greater extent and support enhanced muscular hypertrophy with exercise training merits further investigation.

Finally, the inclusion of MA aged ~70 years is particularly interesting, as at this life-stage, untrained individuals would typically have undergone a notable degree of age-related deterioration in physiological function. However, the MA cohort in this study have maintained extraordinary physiological function, similar to the YC. Whereas, the physiological function has drastically decline in the OC. It is often observed that decrements in physiological function are hastened beyond the age of 80 years, evidenced by the severe deterioration of world record performances previously identified (Lazarus and Harridge, 2017). Therefore, the study of MA beyond this age may provide important insights into the physiological and muscle-specific benefits of continued structured exercise training, or perhaps MA deteriorate more rapidly beyond this point and close the gap between untrained counterparts. However, it should be noted that with the age of MA competition beginning at ~35 years, it is likely that very few MA exist above this threshold and recruitment of these individuals for invasive research studies would be difficult to overcome.

In summary, Chapter 3 provides a deeper, mechanistic insight into the role that chronic endurance training has in offsetting the deterioration of function, body composition and a number of skeletal muscle morphological features. As hypothesised, it appears chronic structured exercise training is beneficial on a number of levels, however the lack of a resistance trained MA cohort and young trained groups (both men and women) precludes a number of important insights. Nevertheless, the continuation of exercise training

throughout the ageing process, is undoubtedly important in the maintenance of physical independence, health-span extension and enhancement in quality of life.

5.3. Integrated Muscle Protein Synthesis Rates Are Comparable Between Endurance-Trained Master Athletes and Untrained Older Individuals

MA have maintained structured exercise training throughout adulthood, and as a result demonstrate a superior phenotype (i.e., skeletal muscle function and morphology) compared with age-matched untrained individuals (see previous chapter). However, the dearth of studies focussed on skeletal muscle remodelling in this highly active population needed to be addressed. Therefore, the aim of Chapter 4 was to investigate how skeletal muscle is regulated in MA compared with untrained age-matched individuals, both during basal rested conditions and following an acute bout of resistance exercise; to understand whether any such differences could provide further mechanistic explanation for the superior physiological function and muscle morphology reported in Chapter 3.

In Chapter 4, this work has shown that MA and OC display equivalent rates of integrated MPS (iMPS) during basal conditions, and following a resistance exercise stimulus. Previous studies have demonstrated that basal rates of MPS do not differ between old and young individuals (Volpi et al., 2001). Furthermore, ten weeks of endurance training did not alter basal MPS rates in young individuals (Wilkinson et al., 2008b). As a result, it was anticipated that basal rates of iMPS would be similar between MA and OC. The study carried out in Chapter 4 confirmed our hypothesis, as it was demonstrated that iMPS rates were similar between groups; indicating that the superior phenotype commonly observed in MA is not reflective of alterations to basal iMPS. The

potency of resistance exercise in stimulating MPS and the superior skeletal muscle properties of MA observed in Chapter 3 led us to propose that MA would demonstrate a superior skeletal muscle remodelling response to exercise. However, interestingly, both MA and OC increased iMPS to a similar extent over the 48 hours of recovery following a resistance exercise stimulus. This suggests that the superior phenotype reported in MA, in Chapter 3, cannot be explained by alterations to exercise-induced skeletal muscle remodelling. Exercise training has been shown to refine the MPs response to an exercise bout both in magnitude and duration (i.e., the MPS response increases to a greater extent but the duration is shorter) (Tang et al., 2008). This would suggest that chronic training, over many years, would further refine exercise-induced remodelling and subsequently enhance the capacity to rapidly repair skeletal muscle in response to exercise-induced mechanical loading, through the addition of myofilaments to the contractile machinery (Damas et al., 2016). However, the shorter duration of the MPS response previously reported with acute tracer approaches would be captured over a 48-hour iMPS; the inclusion of D_2O as the isotopic tracer in this study allowed assessment of iMPS, over a prolonged period, which may be more reflective of MPS and subsequent muscle mass gain over time. Regardless of whether MA and OC have similar basal and exercise-induced rates of iMPS, it could be speculated that OC do not stimulate this muscle adaptive process (iMPS) to the same extent, or with the same frequency, as MA, and consistently remain within basal levels of iMPS. Combined with equivalent with satellite cell content in MA and OC observed in chapter 3, this provides further evidence that the skeletal muscle remodelling capacity in response to resistance exercise does not differ between groups, but the fact MA repeatedly initiate this adaptive process underpins the superior phenotype reported in Chapter 3. However, it remains unknown whether the muscle

adaptive response differs between OC and MA in response to an endurance exercise stimulus.

Exercise training is known to alter the synthesis of specific muscle sub-fractions (i.e., myofibrillar vs. mitochondrial protein synthesis) (Wilkinson et al., 2008b). The focus of this thesis was to investigate the impact of a resistance exercise stimulus on the remodelling of the contractile component of skeletal muscle (i.e., myofibrillar protein). The recruitment of endurance trained MA and the utilisation of a resistance exercise stimulus, provided a novel exercise stimulus for both cohorts and made it possible to examine how chronic exercise training, in general, impacts skeletal muscle remodelling. The findings reported by Wilkinson et al. (2008) demonstrate that with a comparably short training period, fraction-specific MPS is altered. Specifically, endurance training enhanced exercise-induced mitochondrial protein synthesis, whereas resistance training modified the myofibrillar fraction. The MA included in this study have been training for a period of ~50 years, and it was speculated that the continuation of exercise training for such an extended period could potentially drive further refinement in skeletal muscle remodelling that would, one assumes, plateau at an earlier point in adulthood (i.e. endurance exercise-induced stimulation of mitochondrial MPS and modulation of this process with continued training, must, intuitively, level-off after several years of training). Therefore, it would have been of interest for us to investigate the mitochondrial protein synthetic response to endurance training between MA and OC, to determine whether MA displayed a superior capacity for fraction-specific remodelling of mitochondrial proteins compared with OC, which may have been the case (both the magnitude and time-course of the response).

The blunting of exercise-induced MPS in older compared with younger individuals is frequently reported (Kumar et al., 2009, Brook et al., 2016). Protracted periods of

inactivity, common with advancing age, contributes to the development of age-related muscle anabolic resistance and sarcopenia (Breen et al., 2013). Upon further inspection, the first validation study utilising D_2O to assess MPS utilised a unilateral study design (i.e., exercise vs. non-exercise leg) over 8 days and demonstrated that iMPS rates were ~1.4-1.5 %·day^{-1} in a non-exercised control leg, and ~2 %·day^{-1} in an exercise leg (Wilkinson et al., 2014). Despite, the D_2O dosing strategy being different to our approach in Chapter 4, the rates of iMPS reported in the young cohort from this study are ~0.4 %·day^{-1} greater than reported in the present work, and by others (McGlory et al., 2018), in older individuals. The absence of a young cohort in the current study prevents the identification of muscle anabolic resistance to resistance exercise *per se*. Therefore, it is imperative that future studies address this shortcoming to confirm that, regardless of training status, inherent ageing leads to a reduction in exercise-induced MPS.

The inability to upregulate key intramuscular anabolic signalling markers is often proposed to as a mechanistic explanation for the observation of muscle anabolic resistance in response to resistance exercise. Previously, deficits in anabolic signalling have been shown in acute MPS investigations (Kumar et al., 2009). Here, this work has shown subtle differences between MA and OC, however the acute intramuscular phosphorylation events following exercise are convoluted, and a discordance exists between anabolic signalling responses, MPS and skeletal muscle remodelling over time. In order to provide better insight into acute phosphorylation events following exercise, a unilateral exercise model should be employed to identify difference in the fed and exercise-plus-fed state, over the initial several hours of recovery following exercise cessation.

In summary, Chapter 4 addressed a number of shortcomings of the previous investigation (Doering et al., 2016) and generated novel data that provides important new mechanistic

insight into the impact of chronic exercise training on skeletal muscle remodelling in MA. This work demonstrated that MA and OC display similar rates of iMPS during basal and exercise-induced conditions and this coincide with intramuscular anabolic signalling. However, the absence of a YC group and fraction specific MPS precluded a number of important insights. Contrary to our hypothesis, this work has shown OC retain the capacity to upregulate skeletal muscle remodelling, similar to MA.

5.4. Future Research

Despite the detailed whole-body functional to deeper mechanistic investigation of MA in the current thesis, further examination of this unique cohort is critical to delineate the relative contribution of ageing and inactivity to age-related deterioration. Future studies should endeavour to investigate the extent to which MA deteriorate when compared with a young trained cohort. Furthermore, considerable effort should be made to incorporate a comprehensive analysis of individuals that have undertaken both endurance and resistance training throughout the lifespan; incorporating women and the 'oldest' old. Finally, insight into skeletal muscle remodelling requires the inclusion of young trained and untrained individuals, and insight into fraction specific MPS.

5.5. Conclusions

In conclusion, this thesis has provided a comprehensive summary of the current status of the literature on MA and offered a comprehensive insight into how structured exercise training throughout early adulthood into older age promotes the superior phenotype observed in MA. In addition, this work was the first to have shed light on the skeletal muscle remodelling events that regulate muscle mass in MA during basal and exercise-induced conditions. Finally, this work has shown that, irrespective of training status, older

individuals retain the capacity to upregulate skeletal muscle remodelling in response to exercise.

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6. Appendices

6.1. Example of Search Strategy – Ovid (Medline & Embase)

Exercise

1. Exercise/
2. Exercis*
3. Train*
4. Activit*
5. Physical
6. Sport*
7. Contract*
8. (physical adj (exercise* or exertion* or endurance* or therap* or activit* or conditioning or fitness or train*))
9. (exercise adj (train* or intervention* or protocol* or program* or therap* or regim* or activit* or chronic))
10. (fitness adj (train* or intervention* or protocol* or program* or therap* or activit* or regim*))
11. ((training or conditioning) adj (intervention* protocol* or program* or activit* or regim*))
12. ((endurance or aerobic or cardio*) adj (fitness or train* or intervention* or protocol* or program* or therap* or activit* or regim*))
13. ((weight or strength* or resistance) adj (train* or condition* or exercise* or lift* or program*))
14. ((isometric or isotonic or isokinetic or dynamic or eccentric or concentric or pleiometric or plyometric) adj (action* or contraction* or exercis* or train* or intervention or protocol or condition*))

Age

15. (elder* or old* or ?enarian or aged or ag?ing or senior* or geriatric* or frail) or old age

Muscle

16. Muscle/
17. Skeletal muscle
18. (Musc* adj (mass or size or strength or thick* or growth or enlarge* or area or volume or hypertrophy or function* or morphology or architecture or cross sectional area or CSA or fib* or protein*))
19. (Body adj (composition or fat or mass or weight or size))
20. Sarcopenia or atrophy or muscle loss

Function

21. ((isometric or isotonic or isokinetic or dynamic or eccentric or concentric or pleiometric or plyometric or peak or maximum) adj (one rep* max* or 1RM or MVC or torque or power or strength or handgrip or velocity))
22. ((endurance or aerobic or cardio*) adj (performance or capacity or fitness))
23. Function*

Exercise Duration

24. (Master* adj (athlete*)
25. ((Life* or all-time or highly or largely or hugely or extremely or exceptionally or very) adj (active))

Final Search

26. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
27. 16 or 17 or 18 or 19 or 20
28. 21 or 22 or 23
29. 24 or 25
30. 26 and 15 and 27 and 28 and 29

6.2. Isolation of myofibrillar proteins for FSR

For 30-50mg tissue with single bead homogenization

Overview of procedure:

1. Weigh muscle samples, chip if necessary.
2. Homogenize and separate muscle sub-fractions (sarcoplasmic, myofibrillar).
3. Purify myofibrillar fraction and hydrolyse proteins.
4. Column and reconstitute.
5. Derivatize.
6. Run on mass spectrometer.

STEP 2: Homogenization with single bead homogenizer

Before beginning:

- For each sample, label 2 tubes: a 2ml MYO Eppendorf and a 1.5ml SARC Eppendorf. You can also label a third 1.5ml Eppendorf BCA if you need to run a protein content assay for western blots.
- Make up enough 25mM Tris buffer (can be made in advance and stored for up to a month) and homogenization buffer (must be made fresh the morning of) for the number of samples you plan on running today.
- Take the blocks and beads out of the -20 freezer and allow to warm up for ~10 min (otherwise buffer will freeze).
- Turn on centrifuge and let cool down to 4ºC.

Recipe for 50ml of 25mM Tris buffer, pH 7.2

1. In a 50ml Falcon tube, mix Tris-HCl (175.5mg) and Trizma Base (16.75mg) in 25ml of Milli-Q H_2O.
2. Bring to pH to 7.2. using 0.1M HCl.
3. Bring total volume 50ml with H_2O.
4. Store Tris Buffer at 4ºC for up to 1 month.

Recipe for 10ml of homogenization buffer

1. 8 ml of 25mM Tris buffer from above, pH 7.2.
2. 1 PhosStop Tablet, Roche (4ºC fridge).
3. 100µl TritonX-100 (final 0.5%) (room temperature).
4. 1 complete mini (Roche) protease inhibitor tab (kept in door of 4ºC fridge).
5. Bring to pH to 7.2 on ice, with a stir bar in to be sure it is mixing. The pH will change fast, so use 0.1M HCl and wait 30 seconds between drops.
6. Bring volume to 10 ml with 25mM Tris buffer.

To homogenize:

1. Collect samples from freezer, keep on liquid nitrogen until transferred to buffer.
2. Place 500µl homogenization buffer in the 2ml MYO Eppendorf on ice. Be sure to use the flatter bottomed 2mL Eppendorfs (vs. conical 1.5mL Eppendorfs) or the bead won't reach the bottom of the tube.
3. Place a single bead in the Eppendorf.

4. Place the muscle in the Eppendorf.
5. Place the Eppendorf in the cold blocks and lock it place in the (mixing chamber).
6. Set the gauges to 40 seconds at 20/s frequency (ensuring the muscle is well homogenized).
7. Carefully take the Eppendorf out by pushing from the bottom with a pen.
8. Carefully remove bead. Bead can be washed thoroughly in 70% EtOH, dried and reused.

To separate myofibrillar and sarcoplasmic fractions:

9. Centrifuge the homogenate at 4500 rpm for 10 min at 4°C.
10. Remove supernatant, leaving a little left with the pellet to ensure the pellet is not disturbed, and place it in the Eppendorf labelled SARC for westerns. The pellet contains the myofibrillar fraction - do not disturb this.
11. Place 10µl of the sarcoplasmic fraction and 90µl of Milli-Q H_2O the Eppendorf labelled BCA if you are going to run a protein content assay.
12. Store all samples at -80ºC. Can stop here and continue another day.

STEP 3: Purification and hydrolysis of myofibrillar amino acids

Before beginning:

- Turn on centrifuge and let cool to 4°C. Ensure correct rotor is in place (you need the one that fits Eppendorf tubes).
- Set heating block to 50°C (there is a notch that indicates 50°C on the dial on the heating block in the fume hood).
- Set oven to 110°C. Tell the other labs that the oven needs to stay plugged in. Sometimes others will unplug the oven to use the autoclave.
- Label 1 4mL glass screw-top vial per sample.

To purify:

1. Open Eppendorfs containing myofibrillar pellets and place tubes on ice.
2. To the frozen pellet add 500µl Milli-Q H_20.
3. Vortex 5 seconds.
4. Spin for 10min at 1500rpm at 4°C.
5. Replace tubes on ice. Remove supernatant and discard.
6. To the pellet add 1ml 0.3M NaOH.
7. Vortex 5 seconds.
8. Put in heating block at 50°C for 30min, vortex 5 seconds every 10 min.
9. Spin at 10,000rpm for 5min at 4°C. Collagen is not soluble in NaOH and will collect in a pellet. Note that the collagen "pellet" looks more like a smear, and that for some samples you may not get much, if any at all.
10. Assemble your glass screw-top tubes in a rack with foil at the base to prevent the tubes from falling through. Remove caps.

11. Remove supernatant (which now contains the myofibrillar fraction) and place in 4ml glass screw-top tubes.
12. Add 1ml 0.3M NaOH to pellet.
13. Vortex 5 seconds.
14. Spin for 5 min at 10,000 rpm at 4°C.
15. Remove supernatant and combine with the 1ml of the previous supernatant in the screw tops.
16. Place collagen pellet in fridge if measuring collagen FSR or discard.
17. Add 1ml 1M PCA to screw top (containing myofibrillar fraction). Note: must have cloudy-looking precipitate. ***Do not vortex***
18. Spin 10min at 2500rpm at 4°C.
19. Remove supernatant and discard.
20. Add 1ml 70% ethanol to myofibrillar fraction. ***Do not vortex***
21. Spin 10 min at 2500 rpm at 4°C.
22. Remove ethanol and discard (ensure no fluid is in glass tubes).
23. Add 1ml of Dowex (make sure your Dowex has been cleaned and mixed with 0.5M HCL prior to use) and 1ml of 1M HCL.
24. Vortex thoroughly. The white "pancake" of precipitate will float. Try your best to break this up by vortexing.

To hydrolyse:

25. Place rack of tubes in oven at 110°C for 72 hrs, vortex well every 24hrs. Use paper towel to grip vials when vortexing samples that have just come out of oven or you will burn your fingers.
26. Remove from oven. Samples will have denatured so they can sit at room temperature for a few days/weeks until you are ready to column.

STEP 4: Dowex column clean-up and reconstituting

Reagents needed

- 2M NH_4OH
- 1M HCL
- Dowex (50wx8-200 ion exchange resin)
- pH paper

Equipment needed

- Repeater pipette
- 3ml syringes
- Glass wool
- Styrofoam ice-box and sample rack
- Culture tubes
- Rotor-evaporator

Before beginning:

- Label 1 culture tube per sample. Set aside.

- Set up one column per sample. To make a column, stuff a 3mL syringe with small amount of cotton/glass wool. Fill syringe up to 0.5mL: you want enough cotton to trap the Dowex but enough room to add solutions. Do not pack too tightly.
- Add a bit of water to the bottom of the Styrofoam container to prevent smoking when the acids/bases run through the columns.
- Place rack on top of Styrofoam container and suspend columns from rack.

To column:

1. Add 1.2ml of Dowex resin to all columns
2. Add 2-4ml H_2O to neutralize the HCl that is in the resin. Check with pH paper.
3. Add 2ml of 2M NH_4OH (elutes contaminants)
4. Add H_2O until neutral, 10-15mL or ~ 4 rounds (check with pH paper.)
5. Add 4ml of 1M of HCl (ensure pH of 1)
6. Add samples to each column using a transfer pipette and stirring the sample as you draw it up (the charged/acidic column will retain samples)
7. Add H_2O until neutral, 8-10mL for myo's - check often with pH paper. A slight ring of red is ok.
8. Place the columns on top of culture tubes in order to collect eluent
9. Elute with 4.5mL 2M NH_4OH. Add 2mL at a time because 4.5mL won't fit in the column at once.
10. Gently push a plunger into the tops of the syringes to squeeze out 2-3 more drops. Do not push the plunger in the whole way, or you may push out some Dowex.
11. Dry samples in rotor evaporator:
12. Place samples in roto-vac, ensuring they are balanced.

13. There are 2 switches on the front of the machine: turn the left-hand switch ON, do not touch the right-hand switch.
14. Connect hose to tap. Turn tap on full blast.
15. The top of the roto-vac should develop a seal. This may take a few minutes, but make sure you cannot shift or lift the lid before leaving the room. You should also hear a humming from the machine once it gets going.
16. Place hot pack on lid to prevent condensation from collecting.
17. The more samples you have, the longer they will take to dry. It takes ~30 samples about 6-12 hours to dry. Check them regularly, and do not leave the roto-vac on overnight. If you need to leave and the samples are not fully dry, you can remove the tubes and cover them with parafilm or tin foil (do not use saran wrap) and place in fridge, and continue the following day.

To clean up, pour water on used syringes to neutralize them. They can then be thrown out. Glass vials should go in the glass waste. Liquid in the Styrofoam container should go down the sink with lots of water.

To reconstitute:

Once samples are dry you can reconstitute right away or cover the tubes with parafilm or tin foil and leave in the fridge. To do this:

- add 500µl of 0.1M HCl to **hydrolysed** samples, vortex, and place in an Eppendorf tube.

Derivatize according to samples' needs.

NOTE: If you are shipping to metabolic solutions, add 200µl of reconstituted sample to a crimp top tube. Dry sample under N_2 gas, seal with crimp top, and ship.

6.3. Western Blot Protocol

Gel Preparation (day 1):

1. Clean plates by spraying with 70% ethanol and drying with a towel.

2. Make plate sandwich and align in clamp (thumbnail shouldn't catch).

3. Make separating gel cocktail containing the proper amounts of (see table–each amount makes ~2 gels):

Final acrylamide %	5%	7.5%	8%	8.5%	10%	12.5%	15%
H_2O (mL)	5.75	4.9	4.73	4.56	4	3.2	2.4
4X Tris-SDS pH 8.8 (mL)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
30% Acrylamide solution (mL)	1.65	2.5	2.66	2.83	3.33	4.2	5
10% APS (µL)	100	100	100	100	100	100	100
TEMED (µL)	10-16	10-16	10-16	10-16	10-16	10-16	10-16

- Add ≈3.4 mL of gel to each mini gel with 0.75 mm spacers.

4. Overlay with 100 µL of 70% ethanol.

5. Allow to polymerize ≈20 min.

6. Aspirate the ethanol and wash 2X with water.

7. Make 5% stacking gel:

Final acrylamide %	**5%**
H_2O (mL)	4.67
4X Tris-SDS pH 6.8 (mL)	2
30% Acrylamide solution (mL)	1.33
10% APS (µL)	65

8. Place the combs (15 well or 10 well) in the gel at an angle.

9. Add 10-16 µL TEMED.

10. Pour 1 mL (or enough to fill) at the corner under the comb.

11. Push the comb down at an angle making sure not to trap any bubbles.

12. Allow the gel to polymerize for ≈20 minutes.

Gel Run (day 1):

1. Take out and defrost samples and pre-stained protein marker (STD; from –20°C freezer).

2. Put gels in the gel core unit so that the smaller plate is facing inward.

3. Press the gels down and then the core unit and close the clamps.

4. Pour 1X Running Buffer (RB) until it reaches the level of the top of the gaskets.

5. Remove the combs carefully and straight up as to not alter the lanes.

6. Rinse out the lanes with the 1X RB.

7. Add 4 µL (10 well) or 2.5 µL (15 well) STD and 2-20 µL of sample to the lanes (vortexing before adding; return STD and samples to freezer when finished).

8. Now place the gel core into the gel tank.

9. Fill 1X RB up to halfway up the clamps on the front of the gel core.

10. Put on electrode top (make sure black-black and red-red).

11. Using Power Pac 300 plug in electrodes (black-black and red-red).

12. Run at constant amperage at 23 mA/gel and variable voltage until front leaves the gel.

Transfer (day 1):

1. Approximately 5-10 minutes before gel running is complete, take out tub, four filter papers, two membranes, two black/white cartridges, tray and transfer Buffer (TB).

2. Label both membranes (don't touch with hands!) on upper left hand corner

3. Wet membranes TB.

4. Once gel is done running, turn off Power Pac 300, take out the gel core, dump the 1X RB.

5. Remove the gels from the core.

6. Using the gel wedge, separate the two plates from each other and press the gel down onto the smaller plate.

7. After the 2 plates are separated and gel is on the smaller plate, cut off the lanes.

8. Open 1 black/white cartridge and put in tray with TB.

9. Lay first sponge on the black side of the cartridge.

10. Lay one filter paper on the gel (on small plate), and transfer the gel to the filter paper using the gel wedge.

11. Lay the filter paper plus the gel on the sponge (with the filter paper first on sponge).

12. Lay membrane on the gel, face down (as it is sitting in the transfer buffer) and center first.

13. Lay other filter paper on the membrane.

14. Hold one hand on filter (corners) paper and roll out any bubbles with a pipette in the other hand.

15. Lay other sponge on the filter paper.

16. Close up the black/white cartridge and place in the black and red gel transfer unit, so that the black side of cartridge faces black connection of red and black gel transfer unit.

17. Take 2nd gel from gel core unit and repeat steps 6-16 for other gel so that both black/white cartridges are in the black and red gel transfer unit.

18. Place red and black gel transfer unit into the gel tank.

19. Put in small stir bar and place an ice block behind black and red gel transfer unit.

20. Fill gel tank to rim with TB, using all TB that is in tray and tub (return extra TB to fridge).

21. Put on top (make sure black-black and red-red).

22. Place gel tank on stirring plate and stir at a high velocity.

23. Using Power Pac 200 (or 300), plug in electrodes (black-black and red-red), set constant voltage to 100 V and run for 60 min, amperage should be around 300 mA.

24. While transferring, make 5% milk using 2.5 g dry milk and 50 mL TTBS.

Blocking (day 1):

1. When transfer is finished, turn off Power Pac 200 and take off the top.

2. Return ice block (now liquid) to freezer.

3. Take out black and red gel transfer unit and remove and open both cartridges.

4. Discard the filter papers and gel and place each membrane face up into a small container Ponceau – and image.

5. Return the Ponceau to falcon tube and wash the membrane ~5X with water.

6. Place each membrane face up into a small container with 25 mL of 3% milk solution.

7. Rock slowly for 30-60 min.

8. While rocking, return TB to the fridge, return the stir bar, rinse out the gel tank with water, rinse the sponges and dry flat, and rinse the red and black cartridge.

9. After rocking for 30-60 min, take out milk and rinse with TTBS.

10. Dump out TTBS, add new TTBS, and shake for 5 min.

Primary Antibodies (day 1):

1. Make primary antibody solutions in TTBS, 3% milk in TTBS or 3% BSA in TTBS.

2. Take membranes from small container, set on piece of paper (or plastic) and cut membranes according to molecular weight.

3. Put membrane, protein side up, into a 6 well plastic gel container filled with ≈6-10 mL TTBS – rinse 2x and then wash 3 x 5 min in TTBS.

4. Dump TTBS from 6 well plastic gel container and quickly add enough primary antibody to cover (6-10 mL) for each targeted protein.

5. Rock slowly overnight at 4°C.

Secondary Antibodies (day 2):

1. Pipette out primary antibodies and return to respective tube and immediately put in TTBS.

2. When all wells have been cleared of their primary antibodies and are filled with TTBS, dump out TTBS, and pour new TTBS in.

3. Shake for 5 min, dump TTBS, then add new TTBS.

4. Repeat step 3 twice more (for a total of 15 min of washing in TTBS).

5. While washing, make secondary antibodies in 3% milk solution as before or TTBS and add appropriate secondary antibody for each targeted protein.

6. After the wash is complete, dump out TTBS from each well, and add 6-10 mL of secondary antibody and rock slowly for 60 min at RT.

7. After 60 min, dump secondary antibodies, and repeat steps 3-4 for a total of 15 minutes of washing with TTBS again.

8. Make up Chemi (~ approx. 2ML of each solution, totally 4ml) (1:1)), apply to membrane, dry membrane, cover with sheet and image.

9. on computer select western blot, then select ECL and visible marker and image.

References:

- www.fmblab.com